The 8th EURL-AR Proficiency Testing enterococci, staphylococci and E. coli 2010

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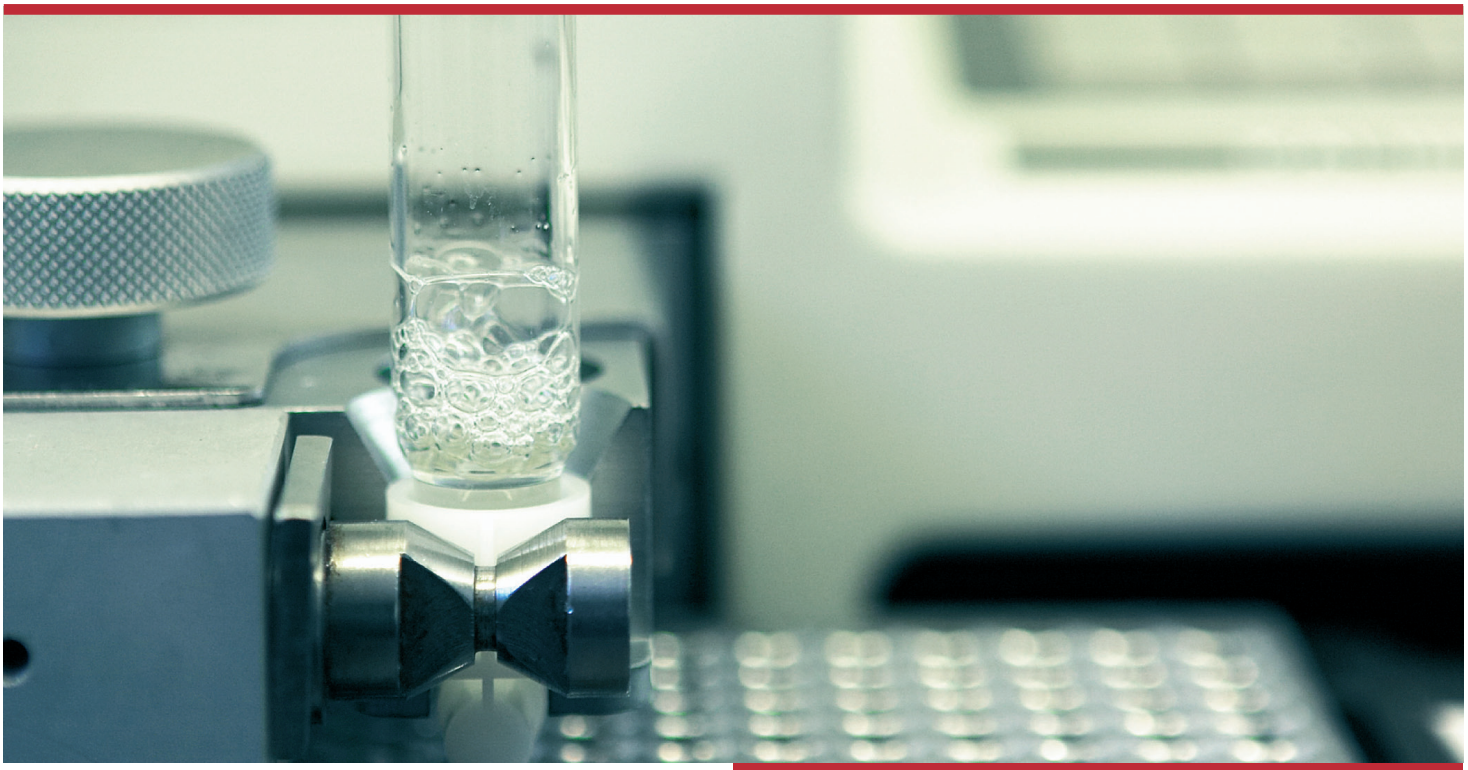

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THE 8th EURL-AR Profiency Testing

Enteroccoci, Staphylococci and *E. coli 2010*

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1. INTRODUCTION

This report summarises the results of the proficiency trial in antimicrobial susceptibility testing (AST) also known as External Quality Assurance System (EQAS 2010) concerning *Escherichia coli*, enterococci and staphylococci. The National Food Institute (DTU Food) was appointed as the European Union Reference Laboratory on Antimicrobial Resistance (EURL-AR) by the European Commission (EC) in 2006. Since then, this has been the 8th EQAS trial carried out on AST within the EURL-AR network. The objective was to monitor the quality of the antimicrobial susceptibility data produced by the National Reference Laboratories (NRL) and to identify areas of interest and/or laboratories, which may need guidance or assistance to produce reliable susceptibility data.

The data in this report are presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential and known only to the EURL-AR and the EU Commission. All conclusions are public.

The technical advisory group for the EURL-AR EQAS scheme consists of competent representatives from all NRL's, who meet once a year at the EURL-AR workshop. During the previous EURL-AR workshops the network agreed upon setting the accepted deviation level for laboratory performance to 5%. As in previous EQASes, incorrect results under a 75% threshold for a test strain/antimicrobial combination have been further analysed in this report, and if no reason was observed that could explain the deviations, the results were subtracted from the evaluation report. Two different MIC tests have been performed at DTU-FOOD in all the selected EQAS strains followed by verification of the obtained MIC results at the United States Food and Drug Administration (FDA), Centre for Veterinary Medicine. Finally, a fourth MIC test was carried out at DTU-FOOD after preparing the agar stab cultures to confirm that the final vials contained the correct strains with the expected MIC values.

Firstly, it is important to stress that the results evaluated in this report are the interpretations of the AST values. As stated in the protocol, the "main objective of this EQAS is to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported to EFSA by the different NRLs", **since the AST data that the Member States (MS) report to EFSA is based on the interpretation of the results, makes sense for the EQAS to evaluate this interpretation.** Secondly, the **participants of an EQAS should evaluate their own results and introduce corrective actions if necessary.** The categorization of an uploaded interpretation as incorrect in the EURL-AR EQAS should in particular cause the participant to perform a self-evaluation. This self-evaluation could very well **include a comment on the fact that the MIC value for strain frequently varies by one dilution either way, in some cases affecting the interpretation of the result.** Even though, the incorrect interpretation based on this dilution difference will still be regarded as a deviation for the overall EQAS reporting, evaluation and in the database, for the self-evaluation, this comment could be an argument to defend the obtained result internally.

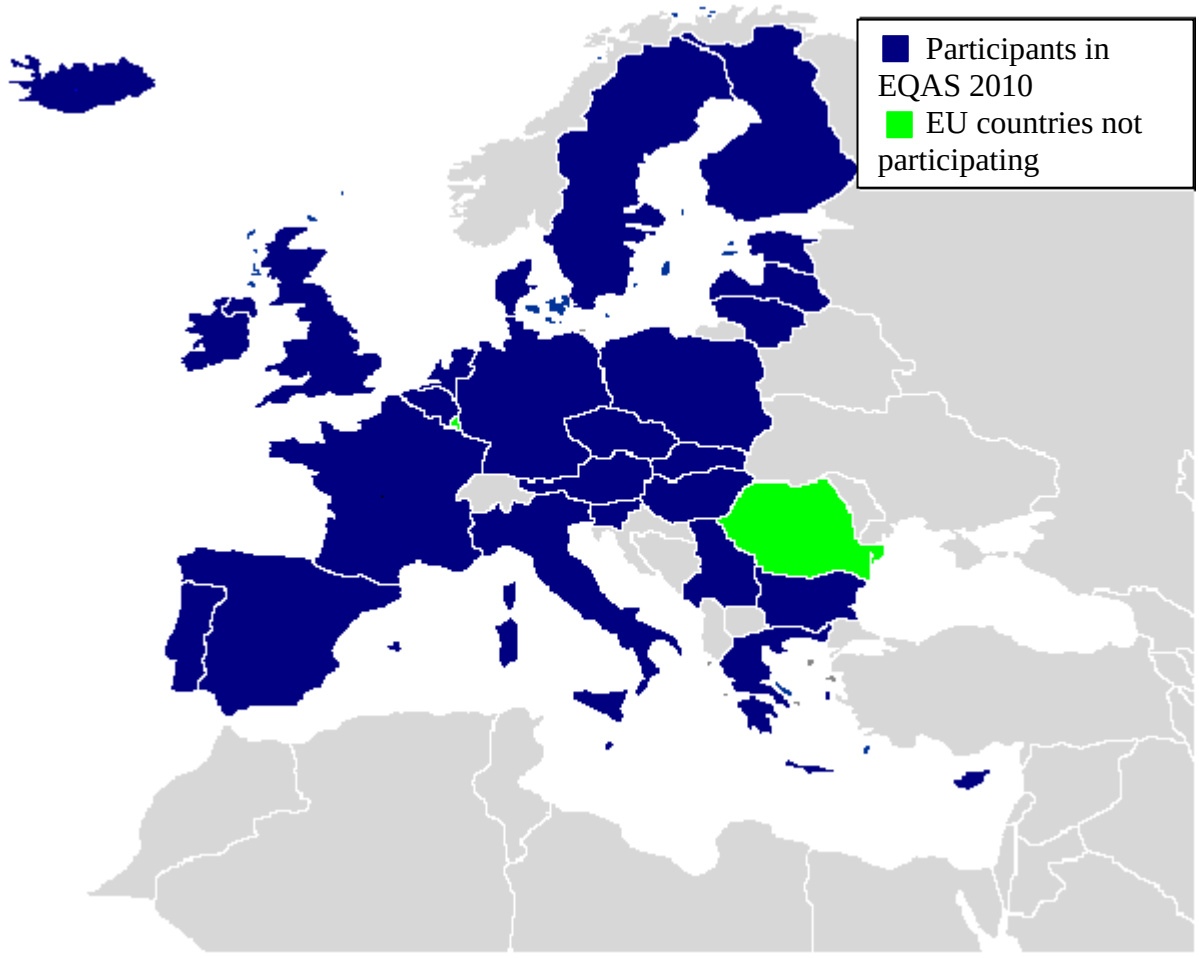
The EURL-AR is accredited by DANAK as provider of proficiency testing; working with zoonotic pathogens and indicator organisms as bacterial isolates (identification, serotyping and antimicrobial susceptibility testing).

2. MATERIALS AND METHODS

2.1 Participants in EQAS 2010

In May 2010, a pre-notification to announce the EQAS 2010 on susceptibility testing for enterococci, staphylococci and *E. coli* was distributed by e-mail to the 32 European NRLs for antimicrobial resistance designated by the MS (App. 1). Seven additional laboratories from Spain, Romania, Denmark, Switzerland, Norway, Serbia and Iceland were enrolled by the EURL-AR to make up a total of 39 participating laboratories, although results from these laboratories have not been included in this report. Participants represented all EU countries except for Luxembourg (App. 2). One of the three NRLs from Spain and the NRL from Romania declined to participate, therefore out of 32 participating laboratories, a total of 30 submitted results (Figure 1). Of those, 22, 28 and 29 laboratories analysed the enterococci, staphylococci and the *E. coli* strains, respectively. Similar number of participation compared to EQAS 2009 when 23, 27 and 28 laboratories submitted results for enterococci, staphylococci and *E. coli*, respectively.

Figure 1. European map illustrating the participating countries in this EQAS trial 2010.



2.2 Strains

Eight strains of enterococci, staphylococci and *E. coli*, respectively, were selected among the DTU Food strain collection. The selection of strains was based on antimicrobial resistance profiles and their MIC values. For quality assurance purposes, three internal control strains have been repeatedly included in every EQAS performed to date, one for each of the bacterial species tested. Antimicrobial susceptibility testing of the strains was performed at DTU Food and the MIC values obtained were used as reference for the EQAS trial (App. 3). In addition, the results obtained were verified by the FDA. After this twofold verification the strains were inoculated in agar stab cultures and subsequently sent to the participating laboratories.

New participating laboratories were provided with the following reference strains, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. Furthermore, they were requested to save and maintain the ATCC reference strains for quality assurance purposes and future EQAS trials.

2.3 Antimicrobials

The panels of antimicrobials used for AST are listed in Table 1.

Table 1. Panel of antimicrobials used for susceptibility testing in each of the organisms examined in the EQAS 2010.

Enterococcal trial	**Staphylococcal trial***	***E. coli* trial**
Ampicillin†	Cefoxitin	Ampicillin†
Chloramphenicol†	Chloramphenicol	Cefotaxime†
Ciprofloxacin	Ciprofloxacin	Ceftazidime
Erythromycin†	Erythromycin	Ceftiofur
Gentamicin†	Florfenicol	Chloramphenicol†
Linezolid†	Gentamicin	Ciprofloxacin†
Streptomycin†	Penicillin	Florfenicol
Quinupristin-dalfopristin†	Streptomycin	Gentamicin†
Tetracycline†	Sulfonamides	Nalidixic acid†
Vancomycin†	Tetracycline	Streptomycin†
	Trimethoprim	Sulphonamides†
		Tetracycline†
		Trimethoprim†

†Antimicrobials recommended by EFSA for monitoring European antimicrobial resistance.
*No specific recommendations have been suggested by EFSA for monitoring resistance in staphylococci.

AST guidelines were set according to the Clinical and Laboratory Standards Institute (CLSI) document – M7-A8 (2009) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Eighth Edition". MIC determination including extended spectrum β-lactamase (ESBL) was performed at the EURL-AR using the Sensititre system from Trek Diagnostic Systems, Magallan Bioscience. The MIC results were interpreted using the epidemiological cut-off values set by EUCAST (www.eucast.org), recommended by EFSA and described in the protocol (App. 4). This year results of the ESBL detection were interpreted according to the recommendations from the EUCAST expert rules.

During the previous years, NRL participants at the EURL-AR workshop have agreed upon harmonising AST analyses by MIC determination using the antimicrobial panel and epidemiological cut-off values recommended by EFSA.

2.4 Distribution

The protocols and other relevant material were made available to all participants from the EURL-AR website (http://www.eurl-ar.eu). In June, cultures were dispatched in double pack containers (class UN 6.2) to the participating laboratories according to the International Air Transport Association (IATA) regulations as UN3373, biological substances category B.

2.5 Procedure

Upon arrival and prior to performing the antimicrobial susceptibility test, the laboratories were instructed to store the tubes in a refrigerator and subculture the strains in accordance with the protocol. The cut-off values for the MIC determination were also listed in this protocol (App. 4, Tables 3.3.1; 3.3.2 and 3.3.3). Participants using disk diffusion method were advised to interpret the results according to their individual breakpoints (App. 5). In both cases the results were categorized as resistant or susceptible. The EURL-AR recommended interpreting intermediate results as susceptible.

The EURL-AR is aware that there are two different types of interpretative criteria of results, clinical breakpoints and epidemiological cut-off values. The terms 'susceptible', 'intermediate' and 'resistant' should be reserved for classifications made in relation to the therapeutic application of antimicrobial agents. When reporting data using epidemiological cut-off values, bacteria should be reported as 'wild-type' or 'non-wild-type' (Schwarz *et al.*, 2010). Due to the different methods of AST used by the participants and also to simplify the interpretation of results, throughout this report, we will still maintain the terms susceptible and resistant, even in the cases where we are referring to wild-type and non-wild-type strains.

The laboratories also entered the zone diameter in millimeters or MIC value of the reference strains. The results were individually compared to the quality control ranges according to the CLSI documents M31-A3 (2008) / M100-S20 (2010), Trek Diagnostic Sensititre System (App. 6).

All participating laboratories were advised to enter the results into an electronic record sheet at the EURL-AR web based database through a secured individual login and password. Alternatively, they were allowed to send the record sheet from the enclosed protocol by fax, mail or email to EURL-AR. The website was opened for data entry until the 15th of September 2010.

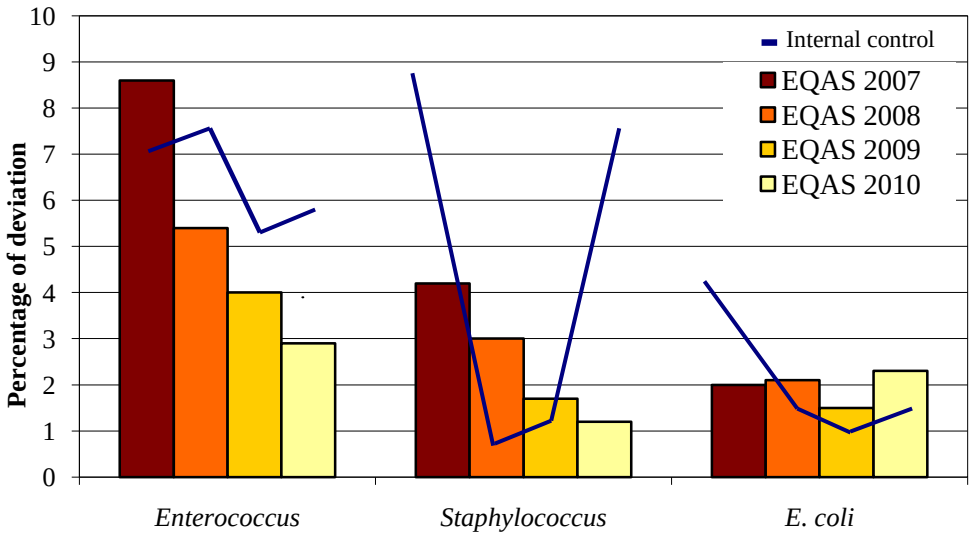
After submitting the data to the secured web site, the laboratories were instructed to retrieve an instantly generated individual report evaluating the submitted results where all deviations from the expected interpretations were reported. In addition and with the aim to improve future EQAS trials, participants were encouraged to fill in an evaluation report generated from the EURL-AR database.

3. RESULTS

3.1 EQAS 2010 versus previous EQASes

Regarding the enterococcal and the staphylococcal trial, the percentages of deviation have been constantly decreasing over the four-year period from 8.6% to 2.9% and from 4.2% to 1.2% respectively (Figure 2). On the other hand, results from the *E. coli* trial showed a slight increase in the deviation percentage from 1.5% obtained during 2009 to 2.3% in the present year. As illustrated in Figure 2, the internal control strains have also followed the increased in deviation, being especially high for the *Staphylococcus* internal control strain.

Figure 2. Comparison of results between previous EQASes and the EQAS 2010 illustrating the deviation levels for the different species tested.



3.2 Deviations by species and method

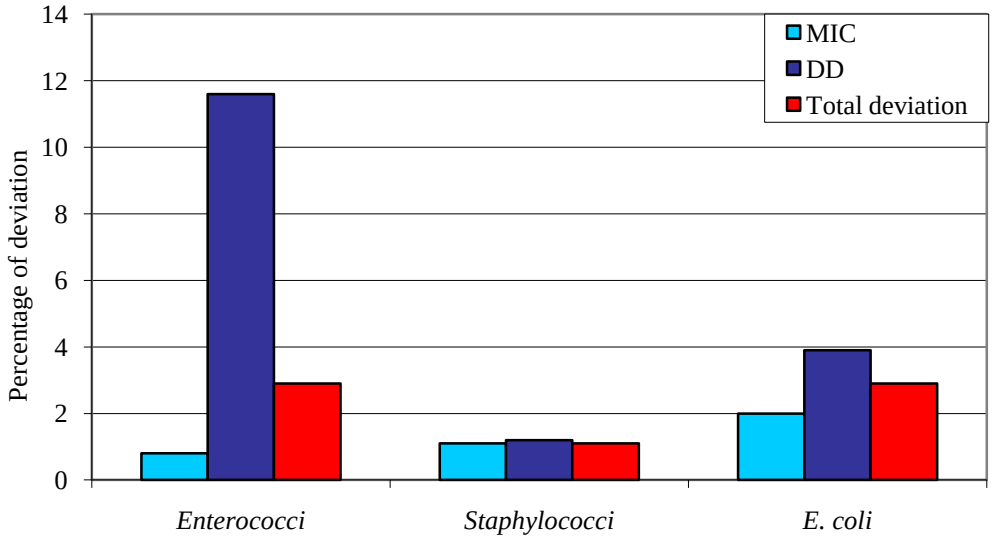
When analysing the data, agar dilution methods and MIC determination have been evaluated together. They are both quantitative methods and the obtained values are the concentrations at which the antimicrobials inhibit the growth of the microorganisms. On the other hand, the ROSCO method used for AST has been considered a disk diffusion (DD) method, since the antimicrobial would diffuse in the agar in the same way as from a disk.

Analysing deviating results by the different species tested (Figure 3), enterococci were the organisms producing the highest percentage of deviation when compared to the other two species. However, the total deviation percentage for the three species remained lower than 3%. In general,

participants performing disk diffusion methods for AST were the ones to contribute with a higher percentage of deviations to the final outcome, while participants performing MIC produced higher number of correct results. This difference in performance is highly noticeable in the enterococcal iteration, where laboratories performing DD have a percentage of deviation fourteen times higher than those performing MIC methods.

A retrospective analysis of the methods used by participants from 2007 to 2010 showed that the number of participants performing MIC has increased from 15 to 18, 14 to 21 and 15 to 25 for the enterococcal, staphylococcal and *E. coli* trials, respectively. In the same order, the number of laboratories performing disk diffusion has declined from 11 to 4, 17 to 7 and 15 to 4.

Figure 3. Percentage of deviations for the different strains in comparison with the different methods used for AST.



As illustrated in Table 2, the percentage of correct results per strain ranged from 94.5% to 99.6% depending of strain. ENT.4,8 was the strains with the lowest percentage of correct results. The staphylococcal trial was the most successful.

The following sections of this report describe in detail the deviations obtained for each one of the three species in this EQAS carried out in 2010 depending on strain, antimicrobial and laboratory. This report also analyses the results obtained for the quality control reference strains.

3.2.1 Enterococcal trial

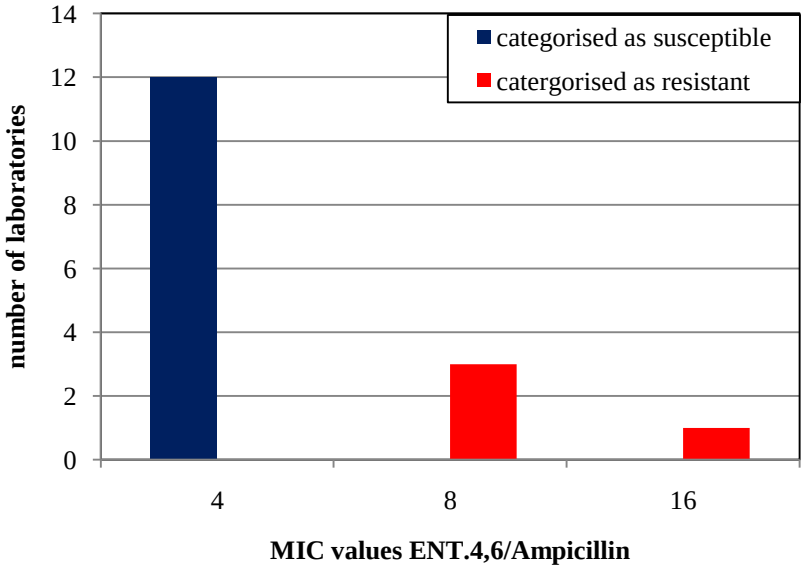
In agreement with previous EURL-AR EQAS's, when the percentage of correct results of a strain/antimicrobial combination was lower than 75%, the data should be further analysed and possible subtracted from the main analysis. The percentage of correct results for the combination of strain ENT.4,6 with ampicillin was below this threshold, therefore results have been excluded from the evaluation. In this particular case, the expected MIC (8 mg/L, resistant) and the cut-off value (> 4 mg/L) to determine if the strain was resistant were within one fold dilution difference. Figure 4 shows the distribution of the different MIC values together with the interpretation of these values obtained by participants performing MIC for the combination of strain ENT.4,6/ampicillin, where

Table 2. The number of AST performed and the percentage of correct results for each strain.

Test strain	AST in total	% correct	Test strain	AST in total	% correct	Test strain	AST in total	% correct
ENT.4,1	200	97.5	ST.4,1	257	97.7	EC.4,1	343	97.1
ENT.4,2	197	96.9	ST.4,2	230	98.7	EC.4,2	342	99.4
ENT.4,3	198	97	ST.4,3	258	98.8	EC.4,3	343	98.5
ENT.4,4	197	98.5	ST.4,4	257	99.2	EC.4,4	344	98
ENT.4,5	199	96.5	ST.4,5	231	98.7	EC.4,5	342	98.8
ENT.4,6	180	98.9	ST.4,6	258	99.6	EC.4,6	315	96.5
ENT.4,7	200	97	ST.4,7	258	98.8	EC.4,7	343	96.2
ENT.4,8	199	94.5	ST.4,8	257	99.2	EC.4,8	319	97.2

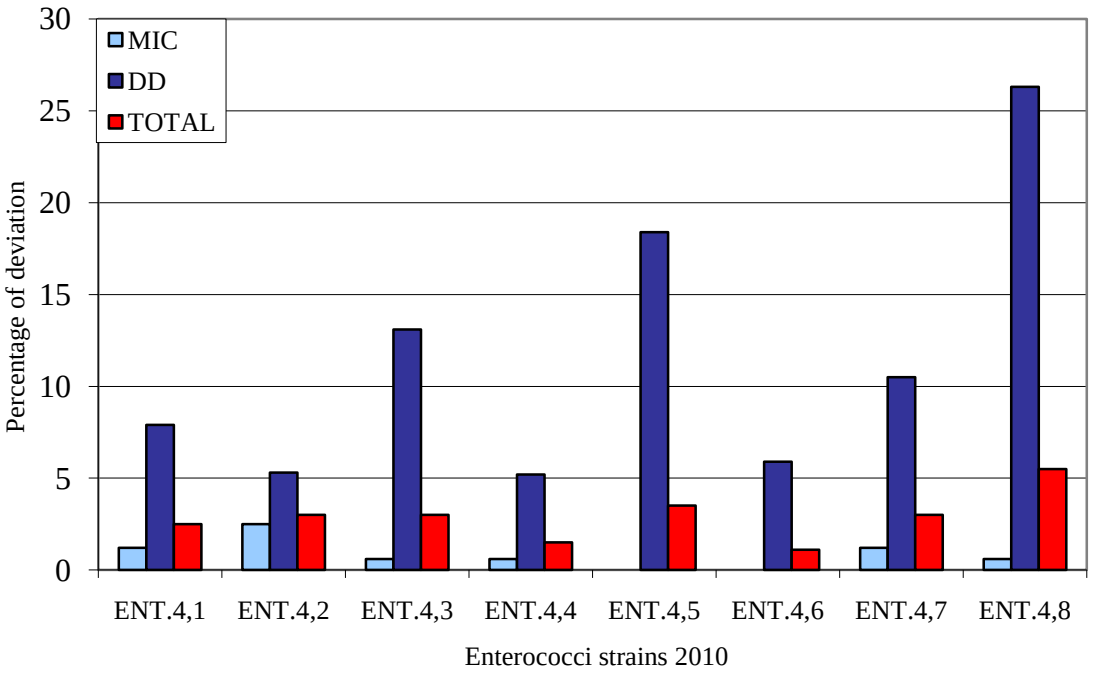
12 participants obtained MIC 4 mg/L, whereas three and one obtained 8 mg/L and 16 mg/L, respectively. Results from four participants performing disk diffusion have been excluded from this particular analysis. However, in Appendix 7a the total percentage of positive results for each strain and antimicrobial tested is presented.

Figure 4. Distribution of the different MIC values obtained by participants performing MIC for the combination ENT.4,6/ampicillin.



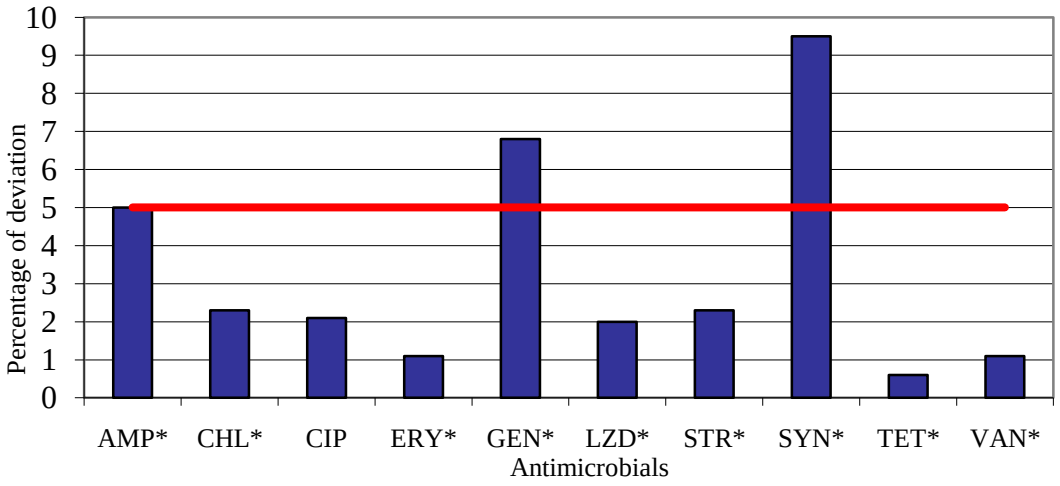
As illustrated in Figure 5, strain ENT.4,8 exhibited the highest deviations in terms of total deviation with a value of 5.5%. In agreement with the results obtained in previous years, the highest percentage of deviation was observed for laboratories performing disk diffusion, and significant differences were obtained when comparing the two methods ($p < 0.01$). Out of the 22 laboratories taking part in the enterococcal trial, 18 performed MIC methods whereas four conducted disk diffusion.

Figure 5. Summary of the deviations obtained per strain according to the method used for AST by all participants.



After discussion with the network (workshop of April 2010), the number of antimicrobials tested against enterococci was reduced from thirteen to ten. Out of those antimicrobials selected in the panel, nine were compounds recommended by EFSA for monitoring antimicrobial resistance across the EU. In this EQAS iteration, synacid, gentamicin and ampicillin were the antimicrobials presenting the highest percentage of deviation with values of 9.5%, 6.8% and 5% respectively (Figure 6). These three antimicrobials belonged to the panel of antimicrobials recommended by EFSA. On the other hand, the percentage of deviation for the rest of the antimicrobials remained lower than 2.3%.

Figure 6. Deviations in enterococcal strains per antimicrobial tested.

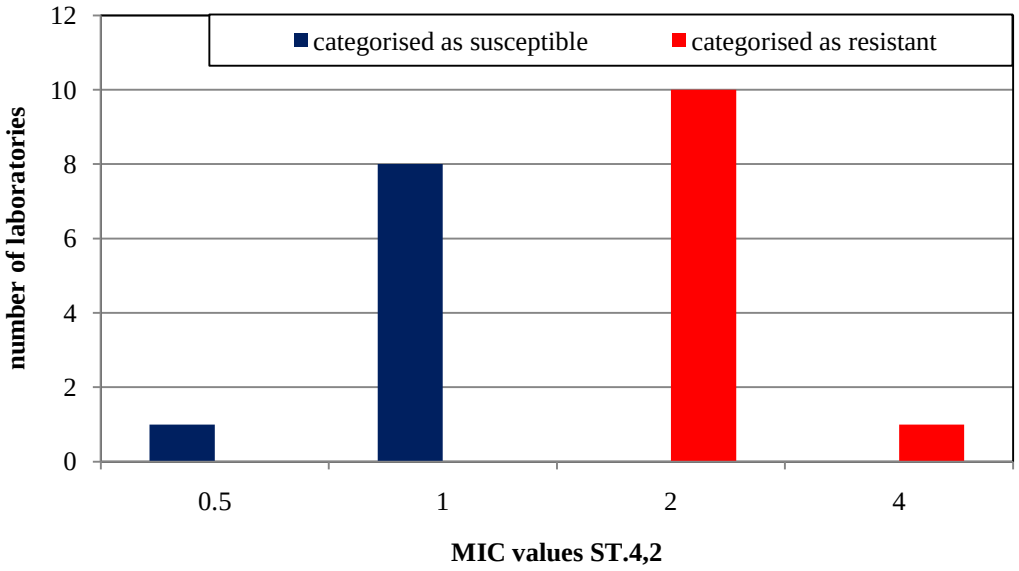


*Antimicrobials recommended by EFSA for monitoring antimicrobial resistance across the EU.

3.2.2 Staphylococcal trial

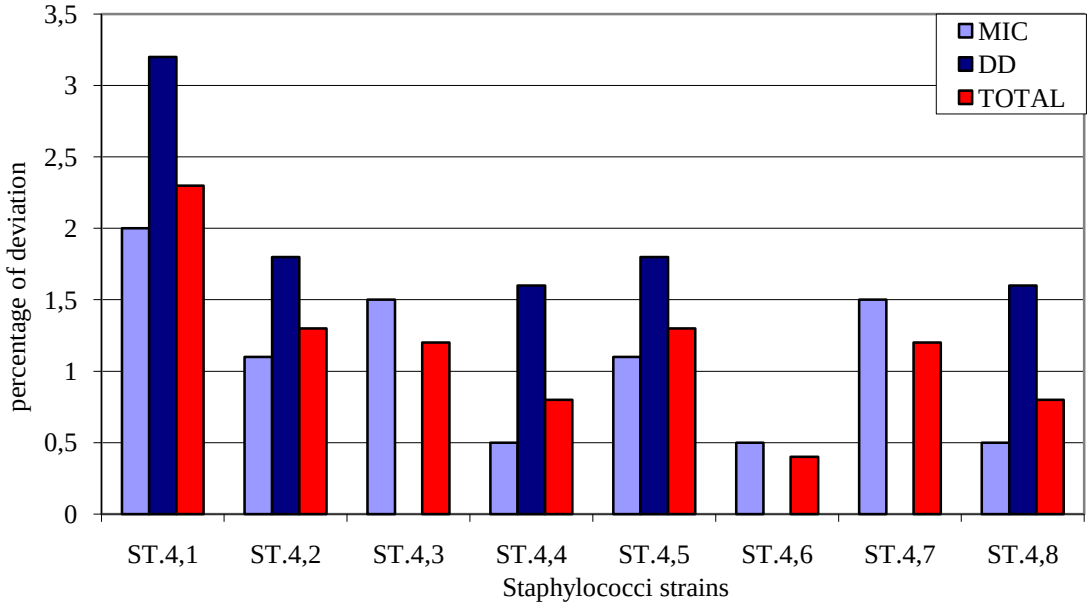
Regarding the staphylococcal strains, two combinations strain/antimicrobial produced more than 25% incorrect results; ST.4,2/ciprofloxacin and ST.4,5/ciprofloxacin. In both cases the expected result for this antimicrobial (MIC = 2 mg/L) and the cut-off value (1 mg/L) to categorise the strains as resistant were within one fold dilution. Thus, producing results within the correct range (± one fold dilution) could conclude in the wrong outcome. These differences in the obtained results appeared to be caused by participants using MIC as well as disk diffusion. These data have been excluded from the report. Figure 7 shows the distribution of the MIC results obtained by the NRL's for the combinations ST.4,2 with ciprofloxacin. In the analysis, only the interpretation of the MIC results reported by the participants performing MIC has been included. The combination ST.4,5/ciprofloxacin is not illustrated in a figure since only two different values were reported, eleven NRL's obtained MIC 1 mg/L and interpreted this value as susceptible against ten NRL's that obtained MIC 2 mg/L and interpreted the result as resistant.

Figure 7. Distribution of the different MIC values obtained by participants performing MIC for the combination ST.4,2/ciprofloxacin.



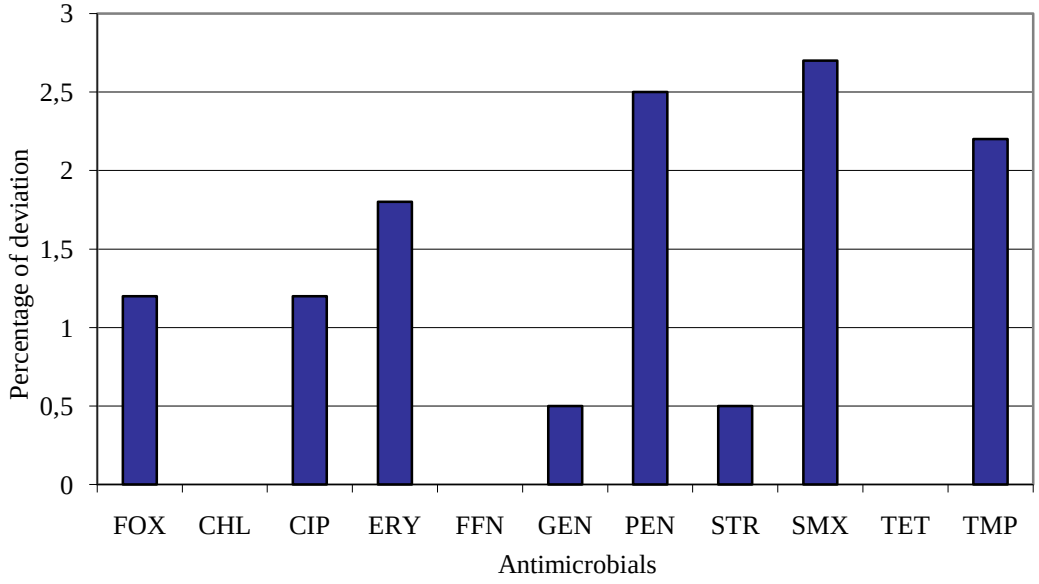
All strains presented deviations lower than 2.3% (Figure 8). Out of 28 laboratories involved in the staphylococcal trial, 21 used MIC methods by comparison to the 16 from the previous year. In addition, five participants performed disk diffusion instead of seven from last year, and two instead of one performed the ROSCO method. Contrarily to results obtained in previous EQAS, no significant differences were observed in results obtained by the two different AST methods ($p = 0.9$).

Figure 8. Summary of the deviations obtained per strain according to the method used for AST by all participants. Results produced by MIC and agar dilution have been evaluated together whereas disk diffusion (DD) has been evaluated together with ROSCO method.



Deviations for all of the drugs were really low and remained below 3% (Figure 9). To see the results generated in the staphylococcal trial with respect to each one of the antimicrobials tested, please refer to appendix 7b.

Figure 9. Deviations in staphylococcal strains per antimicrobial tested.



Methicillin resistant strains.

Among the eight staphylococcal strains selected for the trial, ST.4,1, ST.4,4 and ST.4,5 were confirmed to be methicillin resistant. As it was agreed in the EURL-AR workshop held in Copenhagen 2009, confirmation of *mecA* presence was mandatory for all participants. However,

participant #39 has not reported any results for this part of the test. On the other hand, the rest of the participants performed a total of 211 tests for confirmation of *mecA* in the eight strains with a 100% successful rate.

3.2.3 *E. coli* trial

Regarding the analysis of the *E. coli* data, results obtained for the two combinations of strain/antimicrobial EC.4,6/streptomycin and EC.4,8/florfenicol were lower than 75% with values of 60% and 61.5%, respectively. In both cases, the expected MIC (= 32 mg/L) was one fold dilution above the epidemiological cut-off value (16 mg/L). Results from these combinations have been excluded from the analysis. Figure 10 and Figure 11 show the distribution of the MIC and the interpretation of the values obtained by the NRL's. The interpretations of the millimetres diameters obtained by three participants performing disk diffusion have not been included in any of the two analyses.

Figure 10. Distribution of the different MIC values obtained by participants performing MIC for the combination EC.4,6/streptomycin.

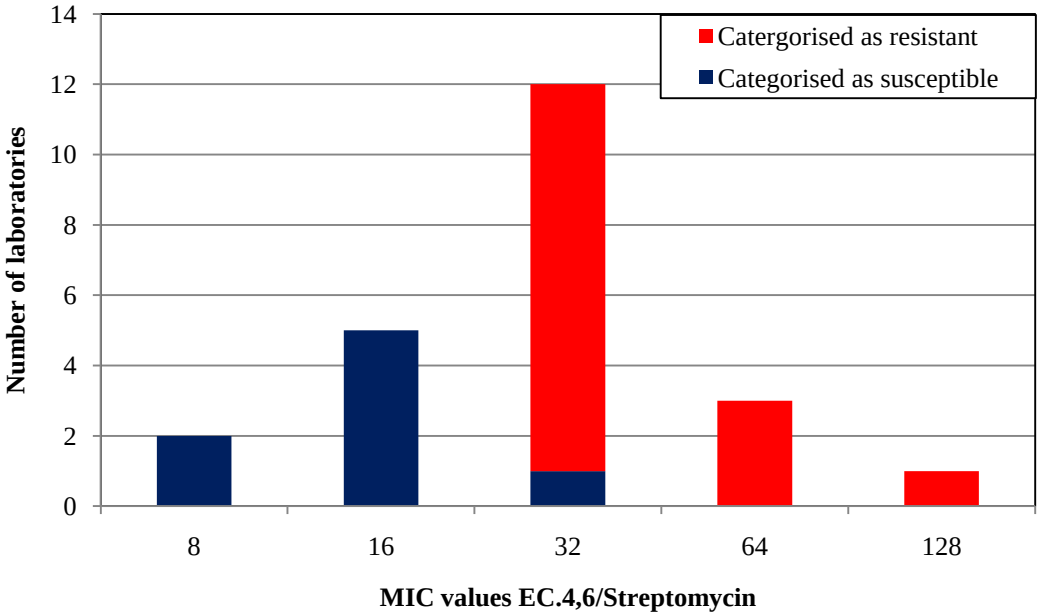
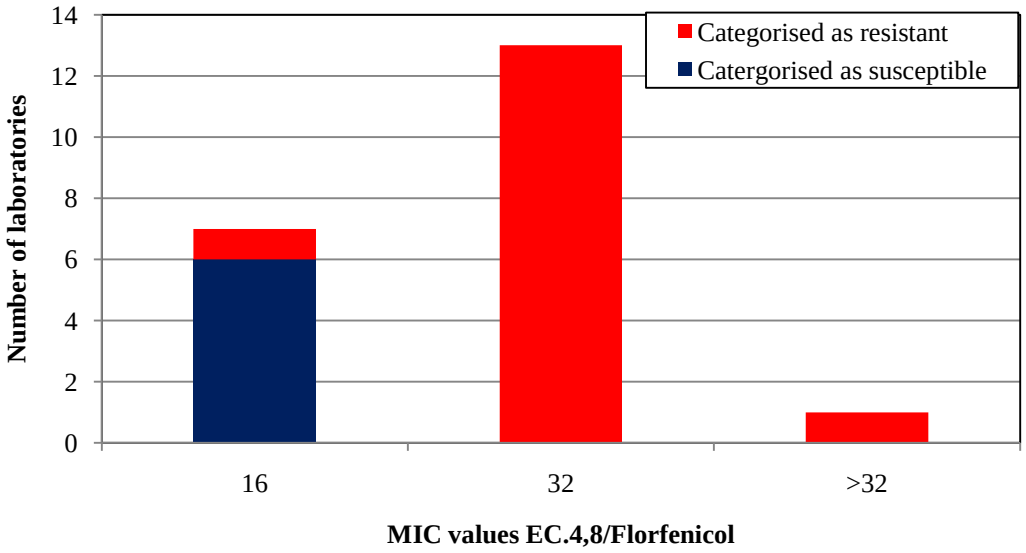
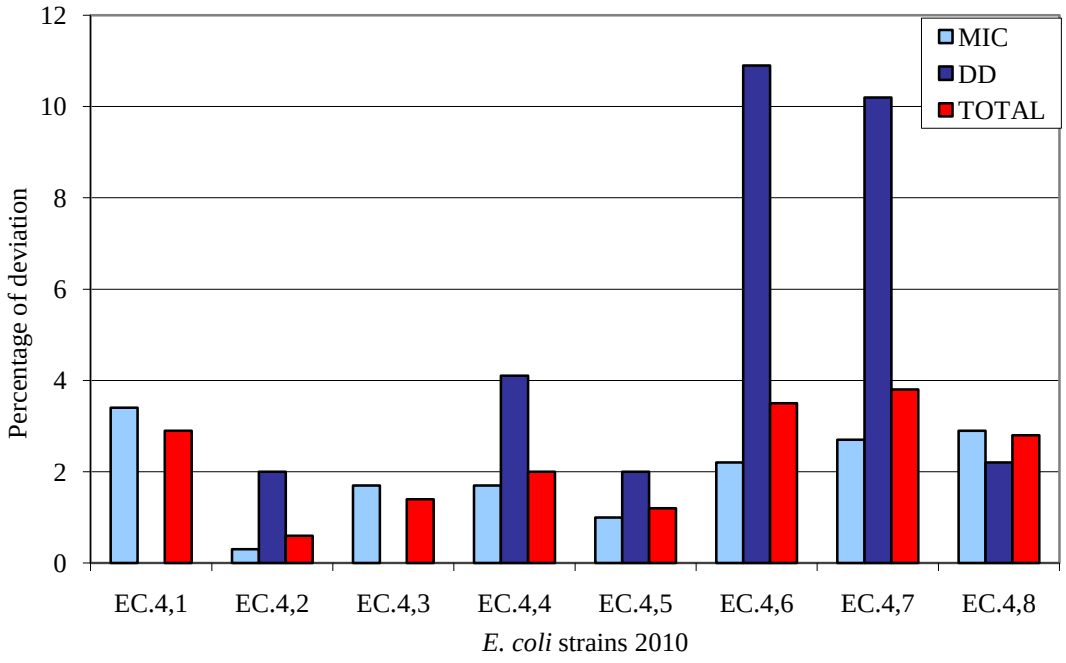


Figure 11. Distribution of the different MIC values obtained by participants performing MIC for the combination EC.4,8/florfenicol.



The deviation values in terms of total deviation ranged between 0.6% and 3.8% depending on the tested strain (Figure 12), with EC.4,7 obtaining the highest deviation percentage (3.8%).

Figure 12. Summary of the deviations obtained per strain according to the method used for AST by all participants.

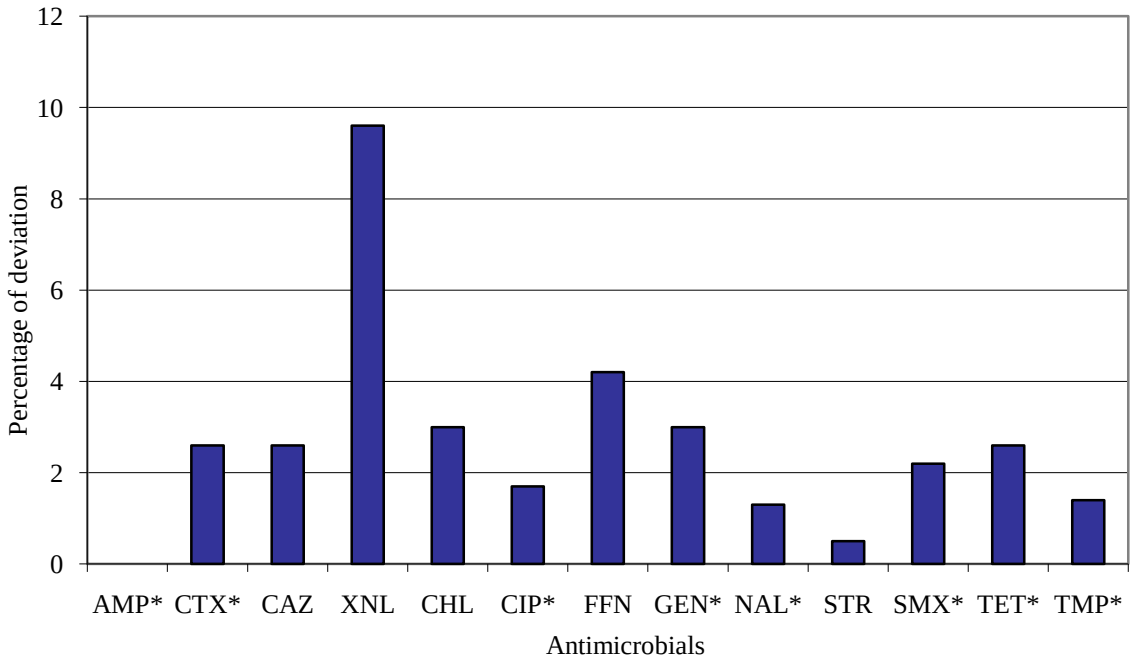


However, laboratories performing disk diffusion achieved a significantly higher percentage of deviation than those participants using MIC methods ($p < 0.01$). Furthermore, for strains EC.4,6 and E.C.4,7 the deviations obtained by laboratories using disk diffusion were four times higher than those obtained by participants performing MIC. This year, the *E. coli* trial was performed by 29

laboratories of which 25 conducted MIC determination and four disk diffusion. For more details in all deviations per antimicrobial, please refer to Appendix 7c.

Figure 13 represents the deviation obtained for each antimicrobial tested. Ceftiofur (XNL) and florfenicol (FFN) were the antimicrobials which have created more difficulties for the NRL's. However, the deviation level for the antimicrobials recommended by EFSA for monitoring antimicrobial resistance remained lower than 3%. For the first time, the deviation obtained for ciprofloxacin was really low (1.7%) resulting in a great improvement when compared with results from previous years. Strains EC.4,4, EC.4,6 and EC.4,8 exhibited low level of resistance to ciprofloxacin with values of 0.5 mg/L, 0.5 mg/L and 0.25 mg/L, respectively. Deviations in these strains were mainly caused by laboratory #40 performing disk diffusion that categorised those strains as susceptible instead of resistant (cut-off value for ciprofloxacin is 0.032 mg/L).

Figure 13. Deviations in *E. coli* strains per antimicrobial tested.



*Antimicrobials recommended by EFSA for monitoring antimicrobial resistance across the EU.

Extended spectrum betalactamase (ESBL) producing strains

Following the EUCAST experts rules, in this year's EQAS for cefotaxime (CTX), ceftazidime (CAZ) and ceftiofur (XNL), MIC values and interpretation for these antimicrobials should be reported as found. The highest deviation was obtained for ceftiofur, with a value of 9.6% mainly caused by participant #39 performing MIC. This participant produced six out of eight results incorrectly without a plausible explanation.

Out of the eight *E. coli* strains selected for the EQAS 2010, EC.4,5 and EC.4,8 were "true ESBL's" and harboured $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ genes, respectively. In addition, EC,4.7 yielded the $bla_{\text{CMY-2}}$ gene and therefore it was an ampC strain. Confirmation of ESBL producing strains is a mandatory part of this proficiency test and 28 out of 29 laboratories carried out the required tests. However, participant #39 did not perform any of the confirmatory tests in any of the three strains,

and did not take part in these mandatory tests. For the rest of the participants, nine out of 28 obtained deviations when confirming extended spectrum betalactamase production.

Laboratories #2 and #32 failed to identify the strain EC.4,8 as ESBL producer. Laboratory #32 obtained MIC values≤ 0.12 mg/L and ≤ 0.25 mg/L for cefotaxime and ceftazidime, respectively instead of 4 mg/L and 32 mg/L, therefore confirmatory tests were never performed. In the case of laboratory #2, the antimicrobials listed in their panel to test against *E. coli* only contained one cephalosporin; cefotaxime. Laboratory #2 obtained a MIC of 0.12 mg/L instead of 4 mg/L and although the participant performed the two confirmatory tests on the strain, both of them were negative for ESBL production.

However, laboratory #2 has contacted the EURL-AR regarding the ESBL results for EC.4,8. This NRL intended to use the EQAS strains to evaluate their Vitek system, they tested EC.4,8 in parallel with Vitek and disk diffusion and they obtained exactly the same results: CTX ≤ 1mg/l, CAZ ≤ 1mg/L, cefepim ≤ 1mg/L, gentamicin ≤ 1mg/L by Vitek and CTX 33mm, CAZ 28mm, gentamicin 20mm by disk diffusion. After obtaining the EQAS evaluation, they went back to the frozen vial sent by the EURL-AR and they performed MIC testing which revealed the following MIC values: CTX 128 mg/L (resistant) and gentamicin >32mg/L (resistant). Since $bla_{\text{CTX-M-15}}$ is generally plasmid mediated, a possible explanation for these results may be the lost of the plasmid harbouring the CTX gene together with an amonoglycoside modifying enzyme during processing of the strain. However, further studies should be carried out to clarify this issue.

A total of seven participants obtained deviations when dealing with the ampC strain EC.4,7. Three laboratories, #15, #22 and #32 confirmed this strain to be ESBL producer together with ampC. The three laboratories reported results for cefoxitin in agreement with the expected MIC (> 16 mg/L or < 14 mm), and therefore they identified EC.4,7 as ampC. For some unknown reason, participant #15 did not perform confirmatory test for ESBL but still identified EC,4.7 as ESBL producer. On the other hand, laboratory #22 obtained an increase in the diameters (≥ 5mm) when performing the two confirmatory tests (CAZ:CAZ/CL and CTX:CTX/CL) and reported the strain as ESBL producer. Participant #32 also reported an increase in the MIC ratio but only for one of the confirmatory test (CAZ/CL:CAZ). Finally, the remaining four participants did not confirm EC.4,7 as an AmpC positive strain. Participants #24 and #30 obtained the correct results for all tests (cefoxitin and the two confirmatory tests) but failed to interpret the results correctly. Participant #29 obtained a susceptible value for cefoxitin and did not find synergy when performing the confirmatory test (CTX/CL:CTX). Participant #40 reported EC.4,7 as susceptible for the two cephalosporins tested; therefore they did not suspect this strain to be cephalosporin resistant. In addition, participant #44 identified strain EC.4,1 as an ampC. They obtained MICs of 1 mg/L, 2 mg/L and >16 mg/L for ceftazidime, cefotaxime and cefoxitin, respectively and they did not find synergy in one of the ESBL confirmatory tests (CAZ/CL:CAZ).

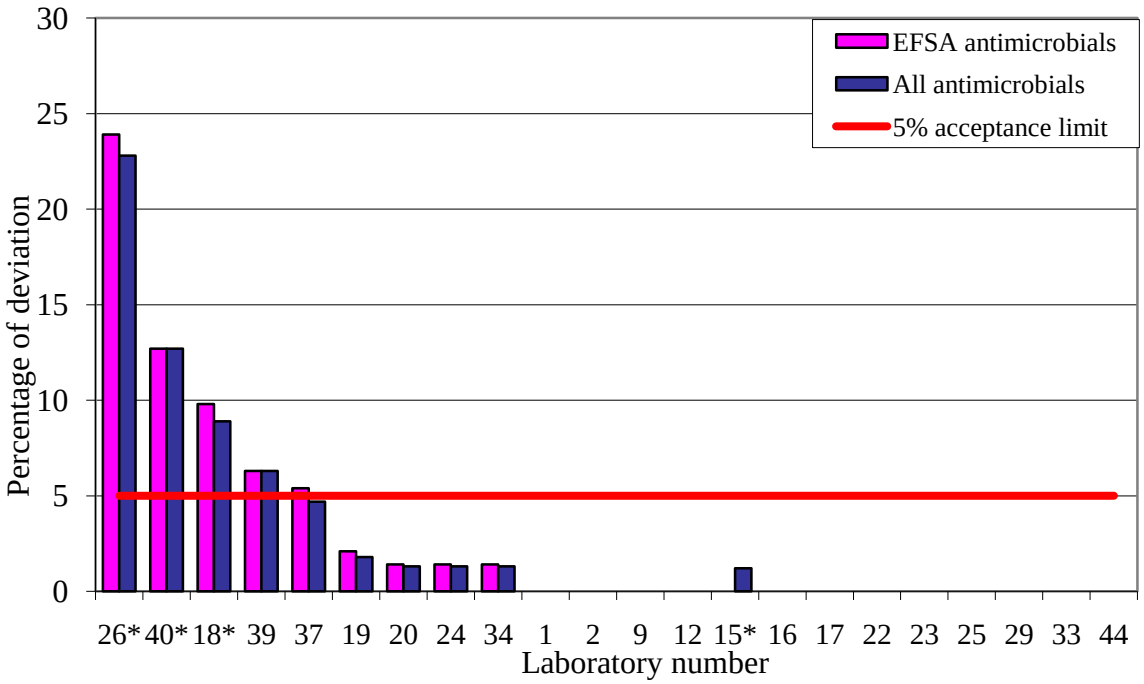
3.3 Deviations by laboratories

3.3.1 Enterococcal trial

When analysing laboratory performance to only those antimicrobials reported to EFSA, out of the 22 participating laboratories, five obtained deviations greater than 5% acceptance limit (Figure 10). This number decreased to four when including all antimicrobials tested. The percentage of deviations differed widely between the laboratories, with a maximum of 23.9% and a minimum of

0% (Figure 14). Three out of the four laboratories performing disk diffusion obtained deviation greater than the 5% threshold. For laboratory #26 the deviations appeared to be caused mainly by three antimicrobials, chloramphenicol, gentamicin and synacid. All susceptible strains were reported as resistant. Laboratory #40 seemed to have difficulties testing against ampicillin, gentamicin and linezolid and also reported most of the susceptible strains as resistant. Participant #18 deviated in gentamicin and synacid with the same issue, reporting of susceptible strains as resistant strains. Finally, participant #39 failed to produce correct results in five different antimicrobials and in all cases they reported all resistant strains as susceptible.

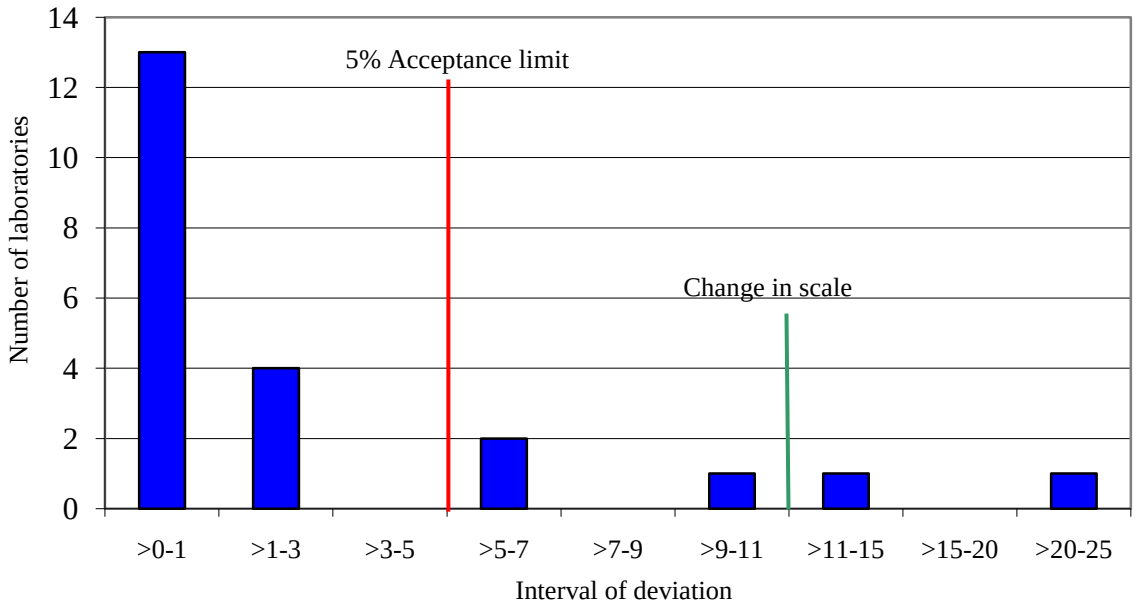
Figure 14. Individual deviations per laboratory in percentage of their total number of enterococcal tests. The laboratories were ranked by decreasing percentage of deviations in accordance with deviations obtained in antimicrobials recommended by EFSA.



*Laboratories performing DD for AST

As shown in Figure 15, a total of 17 laboratories out of the 22 taking part in the enterococcal trial, achieved the acceptance level of performance lower than 5%. On the other hand, five of the participants obtained deviations higher than the agreed threshold. One of these participants fell in the interval of deviation between 21% and 25% and therefore was identified as outlier. However, Appendix 8a summarises all deviations per laboratory.

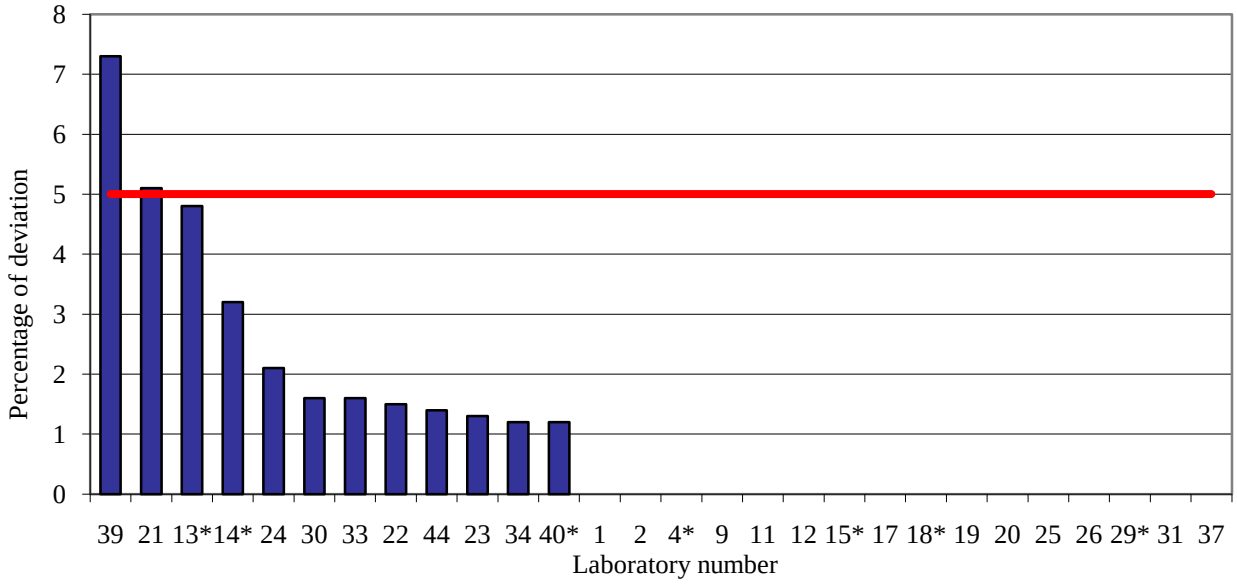
Figure 15. The number of laboratories listed in intervals of percent of total deviations in antimicrobials recommended by EFSA. The vertical line marks the acceptance limit set by the EURL-AR at 5%.



3.3.2 Staphylococcal trial

In this EQAS staphylococcal trial, two laboratories exceeded the 5% acceptance limit of deviation (Figure 16). Furthermore, 16 laboratories performed 100% correctly. Deviations for participant #39 were mainly caused for trimethoprim. In three occasions they obtained resistant values instead of susceptible. Laboratory #21 performing MIC for AST has obtained susceptible values for penicillin instead of resistant values. Appendix 8b shows in detail the deviations per laboratory.

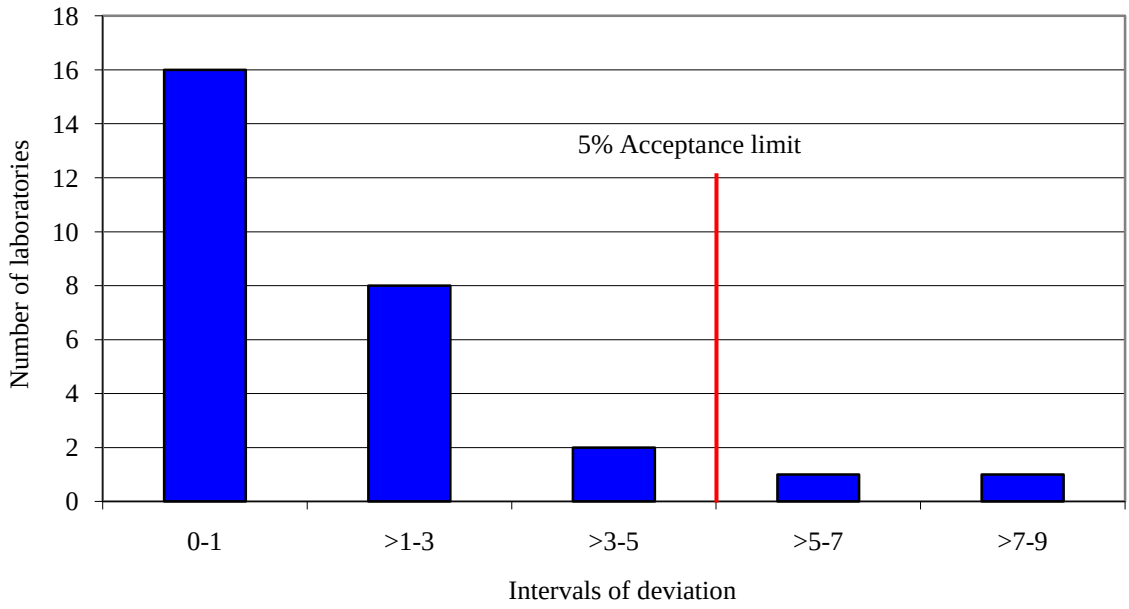
Figure 16. Individual deviations per laboratory in percentage of their total number of staphylococcal tests. The laboratories were ranked by decreasing percentage of deviations.



*Laboratories performing DD for AST

When clustering the laboratories in intervals of deviation as illustrated in Figure 17, the majority of the laboratories clustered in the intervals between 0% and 1% deviations. No outliers were identified in this staphylococcal trial, but the participant that did not perform confirmation of *mecA* cassette will be contacted by the EURL-AR in the near future.

Figure 17. The number of laboratories listed in intervals of percent of total deviations. The vertical line marks the 5% acceptance limit set by the EURL-AR.

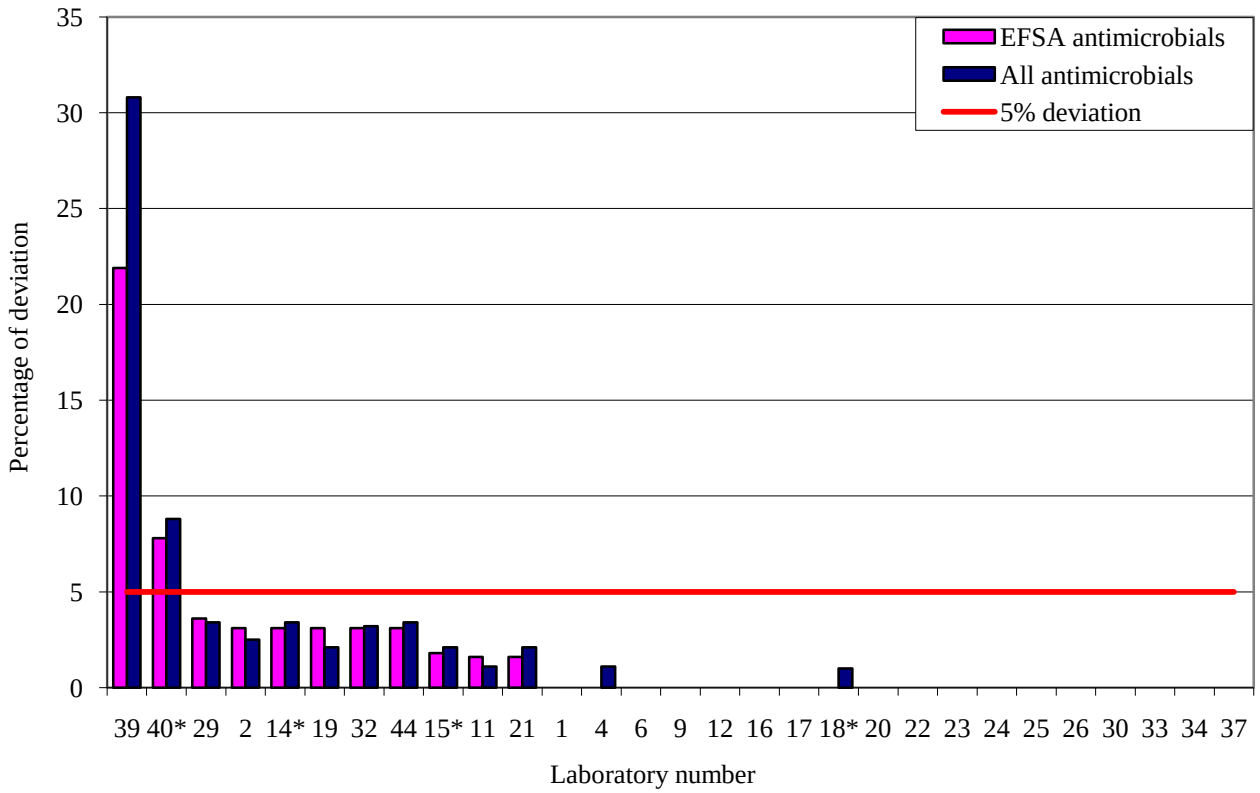


3.3.3 *E. coli* trial

Analysis of results based only on those antimicrobials recommended by EFSA (Figure 18) showed that out of the 29 participating laboratories, two obtained deviations above the stipulated 5%. For laboratory #39, with the highest deviation in this trial, the 21.9% deviation was caused by six different antimicrobials, cefotaxime, chloramphenicol, gentamicin, sulfamethoxazole, tetracycline and trimethoprim. For participant #40, the deviations were caused mainly by two antimicrobials, ceftazidime and ciprofloxacin. In addition, when analysing results including all the antimicrobials tested instead of just those recommended by EFSA, the deviation percentage of laboratory #39 increased considerably from 21.9% to 30.8%.

A total of 18 laboratories obtained 100% of correct results in the antimicrobials reported to EFSA, whereas when including all antimicrobials in the panel, the number of participants performing 100% decrease to 16. To see the deviations for each individual laboratory, please refer to Appendix 8c.

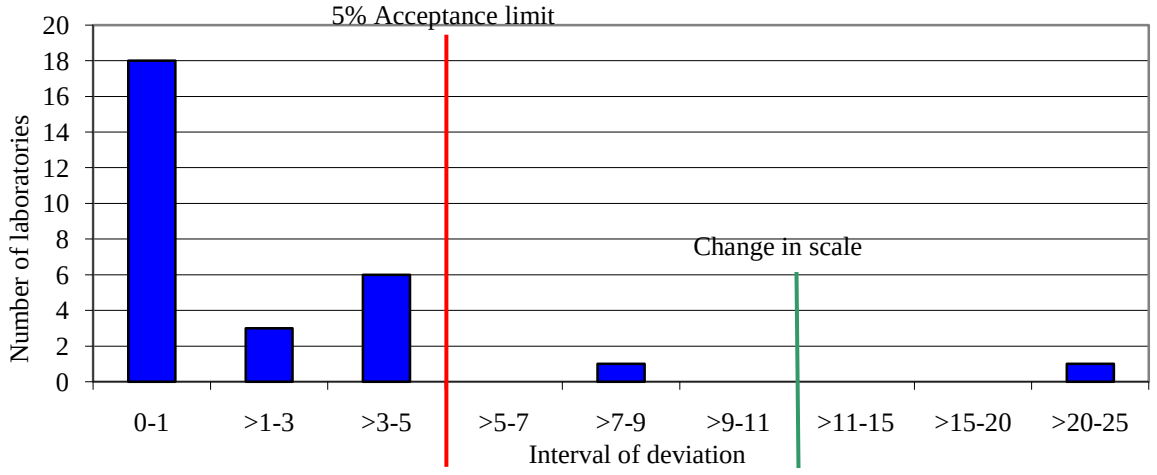
Figure 18. Individual deviations per laboratory in percentage of their total number of *E. coli* tests. The laboratories were ranked by decreasing percentage of deviations in accordance with deviations obtained in antimicrobials recommended by EFSA.



*Laboratories performing DD for AST

As illustrated in Figure 19, the majority of the laboratories obtained deviations in the interval between 0% and 1%. Only two laboratories clustered outside the 5% threshold. For this *E. coli* trial, only one participant was identified as outlier. Appendix 8 is a summary of all the deviations obtained per participating laboratory.

Figure 19. The number of laboratories listed in intervals of percent of total deviations. The vertical line marks the 5% acceptance limit set by the EURL-AR.



3.4 Deviations by reference strains

As the majority of the participants performing AST by disk diffusion methods have followed CLSI guidelines, the results for the reference strains have been evaluated according to them (the quality control ranges can be found in Appendix 6).

3.4.1 Enterococci

The 17 participating laboratories that carried out MIC determination in the reference strain *E. faecalis* ATCC 29212 obtained 100% of results within range (Table 3). This is a total of 152 correct tests.

As CLSI has not published a QC range for *E. faecalis* ATCC 29212 using disk diffusion, the three laboratories that have entered data for the reference strain performing this method for AST could not be evaluated.

Table 3. Deviations obtained for the reference strain *E. faecalis* ATCC 29212 by MIC determination and the minimum and maximum values reported for each antimicrobial.

***E. faecalis* ATCC 29212**

Antimicrobial	MIC deviations /Total no. of tests	QC range MIC	Min value	Max value
Ampicillin	0/16	0.5 - 2	0.5	2
Chloramphenicol	0/16	4 - 16	4	8
Ciprofloxacin	0/12	0.25 - 2	0.5	2
Erythromycin	0/17	1 - 4	1	4
Gentamicin	0/17	4 - 16	4	≤128
Linezolid	0/14	1 - 4	1	2
Streptomycin	0/17	0-256	32	128
Synacid	0/9	2 - 8	4	8
Tetracycline	0/17	8 - 32	8	32
Vancomycin	0/17	1 - 4	2	4

3.4.2 Staphylococci

A total of five laboratories performed disk diffusion within the reference strain *S. aureus* ATCC 25923. Table 4 shows the results and the deviations obtained per antimicrobial. Only two of the values, one for cefoxitin and one for gentamicin were out of range when compared to the expected results. The total number of tests performed with this reference strain was 45 of which 43 were in range.

In addition, two participant performed ROSCO method within this reference strain and the results have not been included in Table 4, since the quality control values were different to those used for

disk diffusion. One of the participants exhibited a deviation for chloramphenicol tested against *S. aureus* ATCC 25923. These participants performed a total of 15 tests and 14 were correct.

Table 4. Deviations obtained for the reference strain *S. aureus* ATCC 25923 by disk diffusion.

Antimicrobial	Deviation/Total no. of tests	QC range	Min value	Max value
Cefoxitin	**1/5**	23-29	26	**32**
Chloramphenicol	0/3	16-26	18	26
Ciprofloxacin	0/5	22-30	23	29
Erythromycin	0/5	22-30	22	28.5
Florfenicol	0/3	None	20	29
Gentamicin	**1/5**	19-27	19	**30**
Penicillin	0/5	26-37	30	37
Streptomycin	0/4	14-22	14	22
Sulfisoxazole	0/3	24-30	24	30
Tetracycline	0/4	24-34	24	33
Trimethoprim	0/3	19-26	20	26

The 20 laboratories which tested the reference strain *S. aureus* ATCC 25913 conducting MIC and agar dilution methods produced three deviations for chloramphenicol and trimethoprim. This means a total of 169 correct results out of 172 tests performed within this strain (Table 5).

Table 5. Range of obtained values for *S. aureus* ATCC 25913 by MIC determination.

Antimicrobial	Deviation/Total no. of tests	QC range	Min value	Max value
Cefoxitin	0/13	1-4	2	4
Chloramphenicol	**2/18**	2-8	4	**16**
Ciprofloxacin	0/17	0.12-0.5	0.12	0.5
Erythromycin	0/20	0.25-1	0.25	1
Florfenicol	0/9	2-8	4	8
Gentamicin	0/19	0.12-1	0.25	≤2
Penicillin	0/18	0.25-2	0.25	2
Sulfisoxazole	0/8	32-128	32	128
Tetracycline	0/20	0.12-1	0.5	
Trimethoprim	**1/15**	1-4	**0.5**	4

3.4.3 *E. coli*

Four laboratories carried out disk diffusion methods with the reference strain *E. coli* ATCC 25922. The total number of test performed with this strain was 48, of these, eight were out of range (Table 6).

Table 6. Range of obtained values for the reference strain *E. coli* ATCC 25922 by disk diffusion and the minimum and maximum values reported for each antimicrobial.

Antimicrobial	Deviation/Total no of tests	QC range	Min value	Max value
Ampicillin	0/2	16-22	18	20
Cefotaxime	**1/4**	29-35	32	**40**
Cefoxitin	**1/3**	23-29	25	**30**
Ceftazidime	**1/3**	25-32	27	**33**
Ceftiofur	**1/3**	26-31	27	**33**
Chloramphenicol	**1/3**	21-27	22	**28**
Ciprofloxacin	0/4	30-40	34	40
Florfenicol	**1/2**	22-28	23	**33**
Gentamicin	0/4	19-26	20	24.4
Imipenem	**1/2**	26-32	29	**40**
Nalidixic acid	0/4	22-28	25	27
Sulfisoxazole	0/2	15-23	18	23
Tetracycline	0/3	18-25	20	25
Trimethoprim	**1/4**	21-28	**17**	26

Finally, 25 laboratories tested the reference strain using MIC determination. They performed a total of 288 tests of which seven were incorrect causing an average deviation of 2.4%. The deviations of this strain were produced by four antimicrobials, ampicillin, cefotaxime, ciprofloxacin and gentamicin (Table 7).

Table 7. Range of obtained values for the *E. coli* ATCC 25922 using MIC determination and the minimum and maximum values reported for each antimicrobial.

Antimicrobial	Deviation/Total no of tests	QC range	Min value	Max value
Ampicillin	**1/25**	2-8	2	**16**
Cefotaxime	**3/25**	0.03-0.12	0.06	**4**
Cefoxitin	0/6	2-8	2	4
Ceftazidime	0/20	0.06-0.5	0.12	0.25
Ceftiofur	0/3	0.25-1	0.25	0.5
Chloramphenicol	0/24	2-8	4	8
Ciprofloxacin	**2/25**	0.004-0.016	0.008	**0.03**
Florfenicol	0/21	2-8	4	8
Gentamicin	**1/25**	0.25-1	0.25	**2**
Imipenem	0/4	0.06-0.25	0.12	0.25
Nalidixic acid	0/24	1-4	1	4
Streptomycin	0/23	4-16	4	8
Sulfisoxazole	0/17	8-32	16	
Tetracycline	0/24	0.5-2	1	2
Trimethoprim	0/22	0.5-2	0.5	1

4. DISCUSSION

4.1 General overview

Analysing the deviating results for each one of the species tested (Figure 2), we can observe a decrease in the percentage of deviation for the enterococci and staphylococci EQAS during the last four years, reaching in the EQAS 2010 the lowest percentage of deviations. On the other hand, when looking at the *E. coli* trial, the percentage of deviation has slightly increased after 2009, when the results reached the lowest percentage of deviations after a gradual decrease. Furthermore, as illustrated in Figure 2, deviations obtained in the internal control strains for all species have also increased in this EQAS 2010. However, results from Figure 2 should be interpreted with care, since the number of NRL's participating during the years continues to change and the difficulty of the test strains is believed to be increasing over the years. There are still two NRL's that have declined to participate in all enterococci, staphylococci and *E. coli* EQAS conducted to date.

The EURL-AR has worked towards harmonizing methodology between NRL's and has recommended MIC as the gold standard method for AST. In this EQAS trial, a considerable increase in the number of participants performing MIC methods has been observed when compared to previous years, especially for the staphylococci iteration. Also for both species, enterococci and *E. coli*, laboratories performing disk diffusion obtained significantly higher percentage of deviation

when compare to participants performing MIC; therefore, the EURL-AR encourage participants using disk diffusion to harmonise methods towards those that deliver a better quality of results, in the case of these two species, MIC.

4.2 Enterococcal trial

Deviations in the eight enterococci strains varied between 5.5% and 1% depending on strain, and were mainly generated by participants performing disk diffusion for AST rather than MIC. Furthermore, three out of the four laboratories performing disk diffusion obtained deviations higher than the 5% acceptance limit.

Synacid was the antimicrobial exhibiting the highest deviation percentage followed by gentamicin and ampicillin. As these three antimicrobials are recommended by EFSA, the importance of these deviations is slightly higher than those that are not used for the EFSA report.

For synacid, deviations were mainly caused by two participants performing disk diffusion; these were participants #26 and #18. In both cases, they obtained resistant values when the strains were expected to be susceptible. Furthermore, laboratory #26 produced the high percentage of deviating results achieved against gentamicin. This participant obtained resistance values in the six strains that were susceptible.

Overall, it appears that the causes of the deviations are individual NRL's conducting disk diffusion and having encountered some type of methodological problem. When performing this method for AST, it is important to consider the different factors that may influence the results, such as temperature, age and concentration of the antimicrobial disks, volume, moisture and pH of the agar media in the petri dish and the turbidity and density of the inoculum.

The number of laboratories deviating more than the 5% acceptance limit was five, one less than in the previous EQAS 2009. Of those, one participant clustered in the interval between 20%-25% deviation and was considered an outlier. This participant and the one that obtained a 12.7% deviation will be contacted by the EURL-AR in the near future with the aim to identify possible causes of deviations and improve the quality of their results.

This year, the number of laboratories performing 100% correctly has increased to 13 from nine in 2009. In addition, for the first time, the total deviation for the enterococcal trial falls below 4%. Also MIC determination of the quality control strain *E. faecalis* ATCC 29212 showed a total of 100% correct results in all antimicrobials tested. This is a total of 152 correct results. The analysis of the reference strains was used as a quality assessment to monitor the excellence of the laboratory procedures.

4.2 Staphylococcal trial

Contrarily to what has been observed in previous years when participants performing disk diffusion reported significantly better results than those performing MIC, for the first time in this staphylococci iteration, no significant differences were observed between the two AST methods. Furthermore, the number of participants performing MIC has increased from 16 in 2009 to 21 this year, demonstrating a successful implementation of the new method for AST. All of the strains and antimicrobials tested presented deviations below 2.3%.

With regards to detection of methicillin resistance, as in previous years, only participant #39 did not perform the relevant tests to identify potential methicillin resistant staphylococci strains that may

have been included in the EQAS. This participant will be contacted by the EURL-AR to clarify any particular reasons for why this test has never been performed. The rest of the NRLs have conducted a total of 211 correct tests. These results demonstrate that all laboratories within the network have the tools optimized to correctly identify MRSA, and show the quality of the data reported throughout the EU.

Two laboratories clustered outside the 5% limit and most of the participants grouped in the deviation interval between 0% and 1%. No outliers were identified in this staphylococcal iteration.

Few deviations were obtained in the reference strains for both, laboratories performing disk diffusion on strain *S. aureus* ATCC 25923 and laboratories using MIC methods on strain *S. aureus* ATCC 25913. One participant obtained a deviation when testing *S. aureus* ATCC 25923 against cefoxitin. This type of deviation is of high importance, since may induce to identify a particular strain as methicillin resistant. Those laboratories obtaining deviations in the reference strains are recommended to take action and assess possible factors that may have a negative influence on the quality of the results.

4.3 *E. coli* trial

In the present EQAS, participants have obtained slightly higher percentage of deviations than in the previous years' EQASes. High deviation percentages were obtained in three of the antimicrobials tested against *E. coli* strains. However, none of these were antimicrobials recommended by EFSA for monitoring antimicrobial resistance. Deviations in EFSA recommended antimicrobials remained lower than 3% which is a very positive outcome. Having into consideration that three of the test strains exhibited low level resistance to ciprofloxacin, deviations for this antimicrobial were very low which is a great improvement from previous years' results. This is probably due to both, the decrease in the number of laboratories performing disk diffusion and the use of a low concentration of ciprofloxacin (1 µg) in the disks that increases the sensitivity of the assay.

Two laboratories obtained deviations above the 5% acceptance limit and one of them clustered in the interval between 20%-25% deviation. This participant appeared to have difficulties in six different antimicrobials without a particular pattern. However, the results for the reference strains were all correct. This participant has been identified as an outlier and will be contacted by the EURL-AR with the aim to assist in any practicality which may need improvement. For the rest of the participants, they majority clustered in the interval of deviation between 0% and 1% which is a very positive result.

Regarding the reference strain *E. coli* ATCC 25922, the percentage of results within range for all tests performed by disk diffusion was 83.3%, much lower than results obtained by participants performing MIC determination (97.6%). All participants obtaining deviations in the reference strain should revise their methods and also assess if contamination of the reference strain has occurred.

Extended spectrum betalactamase (ESBL)

With regard to cephalosporin antimicrobials including the new ESBL interpretation stated in the protocol, the percentage of deviation for cefotaxime (CTX) and ceftazidime (CAZ) has decreased from previous years.

Two of the EQAS strains (EC.4,5 and EC.4,8) exhibited resistance to cephalosporins and were confirmed to be "true ESBL strains". Two laboratories failed to identify some of these strains, one of them used only one cephalosporin in the panel of antimicrobials to test against *E. coli*. The EURL-AR recommends the use of at least two cephalosporin antimicrobials to help identifying ESBL strains, since they can exhibit susceptibility to some cephalosporins and resistance to others. Still, the two confirmatory tests are crucial tools for final identification of ESBL and should be performed with care. Since the combination disk is a similar procedure to disk diffusion, the same factors should be taken into consideration; those are age and concentration of the antimicrobials in the disks, pH of the media, moisture and volume of the agar and finally density of the inoculum together with good laboratory practice when spreading the bacteria in the petri dish.

For strain EC.4,7 (ampC), seven participants produced deviations in this strain; an extremely high number of participants failing to obtained the correct identification, considering the importance of these type of resistance. As stated in the protocol, "ampC detection can be performed by testing the microorganism to cefoxitin and resistance could indicate ampC." In addition, even though identification of betalactamase production is a mandatory part of the EQAS, one of the participants did not participate.

In all, these ten laboratories are encouraged to revise their procedures for ESBL identification and confirmation, and ensure the implementation of a better detection system.

5. CONCLUSIONS

Although the number of laboratories performing above the 5% acceptance limit remained low compared to previous years, two outliers have been identified, one for enterococci trial and one for the *E. coli* trial. Since one of the aims of the EURL-AR is to give specific recommendations to target the individual difficulties, these participants will be contacted in the near future to assess the causes of the deviations and provide guidelines to improve the results. On the same line, laboratory #39, that has never performed the MRSA and ESBL confirmatory tests, will also be contacted in an attempt to help them establish the methodology to perform these type of analysis.

There is still a significant difference in the quality of results obtained by participants performing MIC when compared to those performing disk diffusion, especially for the enterococcal and the *E. coli* trials. The EURL-AR still encourages participants to harmonize towards MIC methods for AST, since they appear to be more reliable and reproducible.

Regarding ESBL producing *E. coli*, they are still considered a priority area for the EURL-AR. For the last two years the number of laboratories failing to identify the strains resistant to cephalosporins has been remarkably high, especially for the ampC strain. We encourage NRL's obtaining deviating results in these strains to perform a re-test as a training exercise and contact us in case of any doubts in the interpretation of results.

Finally, the EURL-AR is open to any suggestions to make a better forthcoming EQAS and encourage the entire network to bring forward any ideas regarding training courses, improvements and areas for discussion that can expand our knowledge in antimicrobial resistance.

6. REFERENCES

[1] Schwarz S, Silley P, Simjee S, Woodford N, van DE, Johnson AP & Gaastra W. (2010) Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J Antimicrob Chemother 65: 601-604.

DTU Food
National Food Institute

EURL-AR EQAS pre-notification:

EQAS 2010 FOR *E. COLI*, STAPHYLOCOCCI AND ENTEROCOCCI

The EURL are pleased to announce the launch of another EQAS. The EQAS provides the opportunity for proficiency testing, which is considered an important tool for the production of reliable laboratory results of consistently good quality.

This EQAS offers antimicrobial susceptibility testing of eight *E. coli* isolates, eight staphylococci and eight enterococci isolates. Additionally, new participants will be offered the following QC strains: *E. coli* ATCC 25922 (CCM 3954), *E. faecalis* ATCC 29212 (CCM 4224), *S. aureus* ATCC 25923 (CCM 3953) (for disk diffusion) and *S. aureus* ATCC 29213 (CCM 4223) (for MIC).

This EQAS is specifically for NRL's on antimicrobial resistance. Therefore, you do not need to sign up to be a participant. All who receive this pre-notification are automatically regarded as participants.

Participation is free of charge for all NRL's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

Please remember to provide the EQAS coordinator with documents or other information that can ease the parcel's way through customs (eg. specific text that should be written on the invoice). As means of avoiding passing the deadline, we ask you to send us this information already at this stage. For your information, the content of the parcel is "Biological Substance Category B": Eight *E. coli*, eight staphylococci, eight enterococci and for new participants also the QC strains mentioned above. The strains are expected to arrive at your laboratory in June 2010.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE

Shipment of isolates and protocol: The isolates will be shipped in June 2010. The protocol will be provided by e-mail/available on the website (www.crl-ar.eu).

Returning of results: Results must be returned to the National Food Institute by the 1st September 2010. When you enter your results via a password-protected website, an evaluation report of your results will be generated immediately.

EQAS report: When the EQAS is concluded, the data will be collected in an overall report in which it is possible to see all participants' results in comparison. In the report the laboratories will be coded, which ensures full anonymity; only the National Food Institute and the EU Commission will be given access to un-coded results.

Next EQAS: The next EURL EQAS that we will have is on antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* which will be carried out in October 2010.

Any comments regarding the EQAS, please contact me by e-mail (lgmi@food.dtu.dk) or by fax (+45 3588 6341).

Sincerely,
Lourdes Garcia Migura

Participant List

Ent.	Staph.	*E.coli*	Institute	Country
X	X	X	Austrian Agency for Health and Food Safety	Austria
	X	X	Institute of Public Health	Belgium
X	X	X	NRL AR on food, National Diagnostic and Research Veterinary Institute	Bulgaria
		X	Veterinary Services	Cyprus
X	X	X	State Veterinary Institute Prague	Czech Republic
X	X	X	The National Food Institute, Copenhagen	Denmark
X	X	X	DTU Veterinærinstituttet, Aarhus	Denmark
X	X	X	Estonian Veterinary and Food Laboratory	Estonia
X	X	X	Finnish Food Safety Authority EVIRA	Finland
	X		ANSES - Maisons-Alfort Laboratory for Food Safety	France
	X	X	ANSES - Ploufragan/Plouzané Laboratory	France
X	X	X	ANSES - Lyon Laboratory	France
X		X	ANSES - Fougères Laboratory	France
X	X	X	Federal Institute for Risk Assessment	Germany
X	X	X	Veterinary Laboratory of Chalkis	Greece
X	X	X	Central Agricultural Office, Veterinary Diagnostical Directorate	Hungary
X	X	X	University of Iceland	Iceland
X	X	X	Central Veterinary Research Laboratory	Ireland
X	X	X	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
X	X	X	National Diagnostic Centre of Food and Veterinary Service	Latvia
X	X	X	National Veterinary Laboratory	Lithuania
X	X	X	Public Health Laboratory	Malta/UK
X	X	X	Food and Consumer Product Safety Authority (VWA)	Netherlands
X	X	X	Central Veterinary Institute of Wageningen UR	Netherlands
X	X	X	Veterinærinstituttet	Norway
X	X	X	National Veterinary Research Institute	Poland
X	X	X	Laboratorio National de Investigacáo Veterinaria	Portugal
			*Institute for Hygiene and Veterinary Public Health	Romania
X	X	X	National Institute of Research-Development for Microbiology and Immunology “Cantacuzino”	Romania
X	X	X	Institute of Veterinary Medicine of Serbia	Serbia
X	X	X	State Veterinary and Food Institute (SVFI)	Slovakia
	X	X	National Veterinary Institute	Slovenia
	X		Laboratorio Central de Sanidad Animal de Santa Fe	Spain
		X	Laboratorio Central de Sanidad Animal de Algete	Spain
			*C N de Alimentacion. Agencia Espanola de Seguridad Alimentria y	Spain
X	X	X	Complutense University of Madrid	Spain
X	X	X	National Veterinary Institute, SVA	Sweden
X	X	X	Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland
X	X	X	The Veterinary Laboratory Agency	United Kingdom
X	X	X	Health Protection Agency	United Kingdom

Designated NRL by the compentent authority of the member state
Laboratories enroled by the EURL-AR
Not a Member State of the EU
* The laboratory declined to participate

Enterococci test strains and reference values (MIC)

Strain	Species	AMP	CHL	CIP	ERY	GEN	LZD	STR	SYN	TET	VAN
ENT.4,1	*E. faecium*	4	8	4	>32	≤16	2	>2048	8	>32	≤1
ENT.4,2	*E. faecalis*	≤2	≤4	2	>32	512	1	>2048	16	>32	≤1
ENT.4,3	*E. faecalis*	≤2	>64	1	>32	≤16	1	>2048	8	>32	2
ENT.4,4	*E. faecalis*	≤2	>64	1	>32	512	1	>2048	16	>32	4
ENT.4,5	*E. faecalis*	≤2	4	2	1	≤16	1	≤64	8	≤1	2
ENT.4,6	*E. faecium*	8	8	1	>32	≤16	2	>2048	8	>32	≤1
ENT.4,7	*E. faecium*	4	8	1	>32	≤16	2	>2048	4	>32	≤1
ENT.4,8	*E. faecium*	4	4	1	2	32	2	≤64	4	>32	>32

Strain	Species	AMP	CHL	CIP	ERY	GEN	LZD	STR	SYN	TET	VAN
ENT.4,1	*E. faecium*	S	S	S	R	S	S	R	R	R	S
ENT.4,2	*E. faecalis*	S	S	S	R	R	S	R	S	R	S
ENT.4,3	*E. faecalis*	S	R	S	R	S	S	R	S	R	S
ENT.4,4	*E. faecalis*	S	R	S	R	R	S	R	S	R	S
ENT.4,5	*E. faecalis*	S	S	S	S	S	S	S	S	S	S
ENT.4,6	*E. faecium*	R	S	S	R	S	S	R	R	R	S
ENT.4,7	*E. faecium*	S	S	S	R	S	S	R	R	R	S
ENT.4,8	*E. faecium*	S	S	S	S	S	S	S	R	R	R

AMP:ampicillin
CHL: chloramphenicol
CIP: ciprofloxacin
ERY: erythromycin
GEN: gentamicin
KAN: kanamycin
LZD: linezolid
STR: streptomycin
SYN:synacid
TET: tetracycline
VAN: vancomycin

Resistant

Staphylococci test strains and reference values (MIC)

Strain	*mecA*	CHL	CIP	ERY	FFN	FOX	GEN	MRS	PEN	STR	SMX	TET	TMP
ST.4,1	yes	8	0.5	>16	4	8	0.5	yes	8	>64	≤32	>32	≤0.5
ST.4,2		8	2	>16	4	4	1		2	16	≤32	>32	≤0.5
ST.4,3		8	0.5	0.5	4	4	>16		8	8	≤32	≤0.5	>32
ST.4,4	yes	8	0.5	0.5	4	16	≤0.25	yes	8	>64	≤32	>32	>32
ST.4,5	yes	4	2	≤0.25	4	8	>16	yes	>16	>64	512	32	≤0.5
ST.4,6		8	0.5	0.5	4	4	0.5		2	>64	≤32	>32	>32
ST.4,7		8	0.5	≤0.25	4	2	0.5		≤0.06	≤4	≤32	≤0.5	≤0.5
ST.4,8		8	1	0.5	4	4	0.5		4	≤4	64	>32	>32

Strain		CHL	CIP	ERY	FFN	FOX	GEN	MRS	PEN	STR	SMX	TET	TMP
ST.4,1	*S. aureus*	S	S	R	S	R	S	yes	R	R	S	R	S
ST.4,2	*S. aureus*	S	R	R	S	S	S	S	R	S	S	R	S
ST.4,3	*S. aureus*	S	S	S	S	S	R	S	R	S	S	S	R
ST.4,4	*S. aureus*	S	S	S	S	R	S	yes	R	R	S	R	R
ST.4,5	*S. aureus*	S	R	S	S	R	R	yes	R	R	R	R	S
ST.4,6	*S. aureus*	S	S	S	S	S	S	S	R	R	S	R	R
ST.4,7	*S. aureus*	S	S	S	S	S	S	S	S	S	S	S	S
ST.4,8	*S. aureus*	S	S	S	S	S	S	S	R	S	S	R	R

Resistant

CHL: chloramphenicol
CIP: ciprofloxacin
ERY: erythromycin
FFN: florfenicol
GEN: gentamicin
MRS: methicillin resistant
PEN: penicillin
STR: streptomycin
SMX: Sulphamethoxazole
TET: tetracyclin
TMP: trimethoprim

E. coli **test strains and reference values (MIC)**

Strain	AMP	CAZ	CHL	CIP	CTX	ESBL gene	FFN	FOX	GEN	NAL	SMX	STR	TET	TMP	XNL	CAZ/CLV	CTX/CLV
EC.4,1	>32	0.25	>64	0.03	≤0.120		>64		1	≤4	>1024	128	≤2	≤1	≤0.5		
EC.4,2	2	0.25	4	≤0.015	≤0.120		4		≤0.5	≤4	≤64	≤8	≤2	≤1	≤0.5		
EC.4,3	>32	0.25	4	≤0.015	≤0.120		4		2	≤4	>1024	>128	>32	>32	≤0.5		
EC.4,4	>32	0.25	8	0.5	≤0.120		8		≤0.5	>64	>1024	128	>32	≤1	≤0.5		
EC.4,5	>32	2	4	≤0.015	>4	*bla* $_{\text{CTX-M-1}}$	4	4	≤0.5	≤4	≤64	≤8	≤2	≤1	>8	synergy	synergy
EC.4,6	>32	0.125	>64	0.5	≤0.120		16		4	16	>1024	32	>32	>32	≤0.5		
EC.4,7	>32	16	4	0.25	>4	*bla* $_{\text{CMY-2}}$	4	64	1	>64	>1024	128	>32	≤1	8		
EC.4,8	>32	32	>64	>4	>4	*bla* $_{\text{CTX-M-15}}$	32	8	>16	>64	>1024	>128	>32	>32	>8	synergy	synergy

Strain	AMP	CAZ	CHL	CIP	CTX	ESBL gene	FFN	FOX	GEN	NAL	SMX	STR	TET	TMP	XNL	CAZ/CLV	CTX/CLV
EC.4,1	R	S	R	S	S		R	S	S	S	R	R	S	S	S		
EC.4,2	S	S	S	S	S		S	S	S	S	S	S	S	S	S		
EC.4,3	R	S	S	S	S		S	S	S	S	R	R	R	R	S		
EC.4,4	R	S	S	R	S		S	S	S	R	R	R	R	S	S		
EC.4,5	R	R	S	S	R	*bla* $_{\text{CTX-M-1}}$	S	S	S	S	S	S	S	S	R	synergy	synergy
EC.4,6	R	S	R	R	S		S	S	S	S	R	R	R	R	S		
EC.4,7	R	R	S	R	R	*bla* $_{\text{CMY-2}}$	S	R	S	R	R	R	R	S	R		
EC.4,8	R	R	R	R	R	*bla* $_{\text{CTX-M-15}}$	R	S	R	R	R	R	R	R	R	synergy	synergy

AMP: ampicillin
CAZ: ceftazidime
CHL: chloramphenicol
XNL: ceftiofur
CIP: ciprofloxacin,
CTX: cefotaxime
FFN: florphenicol
FOX: cefoxitin
GEN: gentamicin
NAL: nalidixic acid
STR: streptomycin
SMX: sulfamethoxazole
TET: tetracycline
TMP: trimethoprim
CLV: clavulanic acid

*Synergy when CAZ/CLV and CTX/CLV≥ 8
Resistant

PROTOCOL

For susceptibility testing of *E. coli*, enterococci and staphylococci

1 INTRODUCTION

One of the tasks as the EU Reference Laboratory for Antimicrobial Resistance is to organise and conduct an External Quality Assurance System (EQAS) on susceptibility testing of *E. coli*, enterococci and staphylococci. The EC/Ent/Staph EQAS 2010 will include susceptibility testing of eight *E. coli*, eight enterococci and eight staphylococci strains together with susceptibility testing of the reference strains *E. coli* ATCC 25922 (CCM 3954), *E. faecalis* ATCC 29212 (CCM 4224), *S. aureus* ATCC 25923 (CCM 3953) (for disk diffusion) and *S. aureus* ATCC 29213 (CCM 4223) (for MIC).

For new participants of the EQAS who have not already received the mentioned reference strains, these are included in the parcel. The reference strains will not be included in the years to come. The reference strains are original certified cultures and are free of charge. Please take proper care of the strains. Handle and maintain them as suggested in the manual 'Subculture and Maintenance of QC Strains'. Please use them for future internal quality control for susceptibility testing in your laboratory.

Various aspects of the proficiency test scheme may from time to time be subcontracted. When subcontracting occurs it is placed with a competent subcontractor and the National Food Institute is responsible to the scheme participants for the subcontractor's work.

2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of susceptibility testing of pathogens originating from food and animal sources, especially *E. coli*, enterococci and staphylococci. Furthermore, to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported to EFSA by different laboratories on *E. coli*, enterococci and staphylococci and to harmonise the breakpoints used within the EU.

3 OUTLINE OF THE EQAS 2010

3.1 Shipping, receipt and storage of strains

In June 2010, the EU appointed National Reference Laboratories will receive a parcel from the National Food Institute containing eight *E. coli*, eight enterococci and eight staphylococci strains. Reference strains will be included for participants who have not previously received these. All strains are non-toxin producing human pathogens Class II. There might be ESBL-producing strains and MRSA among the selected material. The reference strains are shipped lyophilised, and the test strains are stab cultures. On arrival, the stab cultures must be subcultured, and all cultures should be kept refrigerated until testing. A suggested procedure for reconstitution of the lyophilised reference strains is presented below.

3.2 Suggested procedure for reconstitution of the lyophilised reference strains

Please see the document 'Instructions for opening and reviving lyophilised cultures' on the EURL-AR-website (see www.eurl-ar.eu).

3.3 Susceptibility testing

The strains should be susceptibility tested towards as many as possible of the following antimicrobials by the method used in the laboratory when performing monitoring for EFSA. For MIC, the cut off values listed in tables 3.3.1; 3.3.2 and 3.3.3 should be used. The epidemiological cut-off values allow two categories of characterisation – resistant or sensitive. Participants using disk diffusion are recommended to interpret the results according to their individual breakpoints, categorising them into the terms resistant and sensitive. A categorization as intermediary is not accepted; therefore **intermediary results should be interpreted as susceptible**. Interpretations in concordance with the expected value will be categorised as 'correct', whereas interpretations that deviate from the expected interpretation will be categorised as 'incorrect'.

The cut-off values used in the interpretation of the MIC results are developed by EUCAST (www.eucast.org). Participants performing disk diffusion, please notice that EUCAST has recommended epidemiological cut-off values for interpretation of inhibition zones for some of the antimicrobials.

With regard to MIC range and/or disc content we ask you to fill in these pieces of information in the database. Also, if you ***do not use*** the cut-off values listed in the protocol for interpretation of the susceptibility results, please fill in or update the breakpoints used, in the database.

3.3.1 *E. coli*

Antimicrobials for *E. coli*	MIC (µg/mL) **R is >**
Ampicillin, AMP	8
Cefotaxime, CTX	0.25
Ceftazidime, CAZ	0.5
Ceftiofur, XNL	1
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	0.032
Florfenicol, FFN	16
Gentamicin, GEN	2
Nalidixic acid, NAL	16
Streptomycin, STR	16
Sulfonamides, SMX	256
Tetracycline, TET	8
Trimethoprim, TMP	2

ESBL production

The following tests regarding ESBL production are mandatory: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) should be confirmed by confirmatory tests for ESBL production.

The confirmatory tests for ESBL production require testing with a pure antimicrobial (CTX and CAZ) vs. a test with the same antimicrobial combined with a β-lactamase inhibitor (clavulanic acid). Synergy is defined as a 3 dilution steps difference between the two compounds in at least one of the two cases (MIC ratio ≥ 8, E-test 3 dilution steps) or an increase in zone diameter ≥ 5 mm (CLSI M100 Table 2A; enterobacteriaceae). If the test shows signs of synergy it is an indication of the presence of ESBL.

Confirmatory tests for Metallo beta lactamase require comparison between imipenem (IMI) and IMI/EDTA, synergy is in this test defined as a MIC ratio ≥ 8 or E-test 3 dilution steps difference (CLSI M100 Table 2A; enterobacteriaceae). If the test shows signs of synergy it is an indication of the presence of ESBL.

Additionally, AmpC detection can be performed by testing the microorganism to cefoxitin (FOX), resistance to FOX could indicate AmpC. Verification of AmpC requires PCR or sequencing.

The EURL-AR aim to harmonize with EUCAST expert rules. CONCERNING **CEFOTAXIME, CEFTAZIDIME AND/OR CEFTIOFUR** USED WHEN DETECTING ESBL-PRODUCING STRAINS IN THIS EQAS, MIC VALUES AND INTERPRETATIONS FOR THESE ANTIMICROBIALS SHOULD BE REPORTED AS FOUND.

3.3.2 Enterococci

Antimicrobials for enterococci	MIC (µg/mL) **R is >** *E. faecium*	MIC (µg/mL) **R is >** *E. faecalis*
Ampicillin, AMP	4	4
Chloramphenicol, CHL	32	32
Ciprofloxacin, CIP	4	4
Erythromycin, ERY	4	4
Gentamicin, GEN	32	32
Linezolid, LZD	4	4
Streptomycin, STR	128	512
Quinpristin-dalfopristin (Synacid), SYN	1	32
Tetracycline, TET	4	4
Vancomycin, VAN	4	4

Please find information on the test forms showing which test strains are *E. faecium* and *E. faecalis* respectively.

3.3.3 Staphylococci

Antimicrobials for *S. aureus*	MIC (µg/mL) **R is >**
Cefoxitin	4
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	1
Erythromycin, ERY	1
Florfenicol, FFN	8
Gentamicin, GEN	2
Penicillin, PEN	0.125
Streptomycin, STR	16
Sulfonamides, SMX	128
Tetracycline, TET	1
Trimethoprim, TMP	2

Some of the strains may be methicillin resistant. Testing the staphylococci also include tests regarding methicillin resistance - **confirmation of *mec*A presence is mandatory**. The strains may be tested by any method that you prefer. The result must be uploaded as 'positive' or 'negative'. According to the CLSI recommendations (M100-S19, table 2C), all MRSA should be regarded resistant for all β-lactam antibiotics.

4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the test forms, and enter your results into the interactive web database. Please read the detailed description below before entering your results. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print an evaluation report of your results. Please submit results by latest September, 30, 2010.

If you do not have access to the Internet, or if you experience difficulties entering the data, please return results by e-mail, fax or mail to the National Food Institute.

All results will be summarized in a report which will be made available to all participants. The data in the report will be presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential and known only to the EURL-AR and the EU Commission. All conclusions are public.

If you have any questions, please do not hesitate to contact:

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5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read this passage before entering the web page. Before you go ahead, you need your test form by your side together with your breakpoint values.

You are able to browse back and forth by using the forward and back keys or click on the EURL logo.

You enter the EURL-AR EQAS 2010 start web page (http://thor.dfvf.dk/crl) then write your username and password in low cases and press enter. Your username and password is the same as in the previous EQAS's arranged by The National Food Institute. If you have problems with the login please contact us.

Click on either "*E. coli* test results", "enterococci test results" or "staphylococci test results" depending on your results. The below description is aimed at *Salmonella* entry but is exactly the same as for *E. coli*, enterococci and staphylococci entry.

Click on "Start of Data Entry - Methods and Breakpoints for Salm."

In the next page you navigate to fields with the Tab-key and mouse.

Fill in what kind of method you have used for the susceptibility testing of *Salmonella* and the brand of discs, tablets, MIC trays etc.

Fill in the relevant information, either disk content or MIC range. If you use disk diffusion, please upload the breakpoints used.

Click on "save and go to next page"

In the data entry pages for each *E. coli*, enterococci and staphylococci strain, you enter the obtained value and the interpretation as R or S.

For *E. coli*, you also type in results for the ESBL tests.

If you have not used an antimicrobial, please leave the field empty.

Click on "save and go to next page"

When uploading data on the reference strains please enter the zonediameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to, etc. If you do not use CLSI guidelines for AST on the reference strains, please add a comment on the method used.

Click on "save and go to next page"

This page is a menu, from where you can review the input pages, approve your input and finally see and print the evaluated results:

Browse through the pages and make corrections if necessary. Remember to save a page if you make any corrections. If you save a page without changes, you will see an error screen, and you just have to click on "back" to get back to the page and "go to next page" to continue.

Please fill in the evaluation form.

Approve your input. Be sure that you have filled in all the results before approval, as YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database, but allows you to see the evaluated results.

Breakpoints used in daily routine (disk diffusion) - Enterococci

Antimicrobial	Lab. no.	R≤ mm	S≥ mm
Ampicillin, AMP	15	16	19
	18	16	17
	26	16	17
	40	16	17
Chloramphenicol, CHL	15	19	23
	18	12	18
	23	12	18
	26	12	18
	40	12	18
Ciprofloxacin, CIP	18	15	21
	26	15	21
	40	15	21
Erythromycin, ERY	15	17	22
	18	13	23
	26	13	23
	40	13	23
Gentamicin, GEN	15	11	17
	18	12	15
	40	12	15
Linezolid, LZD	15	24	24
	18	20	23
	26	20	23
	40	20	23
Streptomycin, STR	15	12	14
	18	11	12
	40	11	15
Tetracycline, TET	15	17	19
	18	14	19
	26	14	19
	40	14	16
Vancomycin, VAN	15	17	17
	18	14	17
	26	14	17
	40	14	17

Breakpoints used in daily routine (disk diffusion) - Staphylococci

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Chloramphenicol, CHL	14	22	23
	15	19	22
	18	12	18
	29	12	18
	40	12	18
Ciprofloxacin, CIP	13	15	21
	14	18	22
	15	19	22
	18	15	21
	40	15	21
Erythromycin, ERY	13	16	22
	14	18	22
	15	17	22
	18	13	23
	40	13	23
Florfenicol, FFN	14	18	22
	15	14	19
	18	12	18
Gentamicin, GEN	13	12	15
	14	19	
	15	20	20
	18	12	15
	40	12	15
Penicillin, PEN	13	28	29
	14		29
	15	29	29
	18	28	29
	40	28	29
Streptomycin, STR	13	12	15
	15	13	15
	18	11	12
	40	11	15
Sulfamethoxazole, SMX	13	12	17
	14	11	17
	18	12	17
	40	12	17
Tetracycline, TET	13	14	19
	14	20	23
	15	17	19
	18	14	19
	40	14	19
Trimethoprim, TMP	14	15	20
	18	10	16
	40	10	16

Breakpoints used in daily routine (disk diffusion) - *E. coli*

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Ampicillin, AMP	14		19
	15	15	21
	18	13	17
	40	16	17
Cefotaxime, CTX	14		26
	15	22	26
	18	27	
	40	14	23
Ceftazidime, CAZ	14	20	
	15	18	26
	18	22	
	40	14	18
Ceftiofur, XNL	14		21
	15	17	21
Chloramphenicol, CHL	14		23
	15	18	22
	18	12	18
	40	12	18
Ciprofloxacin, CIP	15	21	25
	18	15	21
	40	15	21
Florphenicol, FFN	14		19
	15	14	19
	18	12	18
Gentamicin, GEN	14		18
	15	15	18
	18	12	15
	40	12	18
Nalidixic acid, NAL	14		20
	15	14	20
	18	13	19
	40	13	19
Streptomycin, STR	15	12	15
	18	11	15
	40	11	15
Sulfamethoxazole, SMX	14		17
	15	11	17
	18	12	17
	40	12	17
Tetracycline,TET	14		19
	15	16	19
	18	11	15
	40	11	15
Trimethoprim, TMP	14		20
	15	11	16
	18	10	16
	40	10	16

Quality control ranges for the control strains

E. faecalis ATCC 29212	
Antimicrobial	**MIC**
Ampicillin, AMP	0.5 - 2
Chloramphenicol, CHL	4 - 16
Ciprofloxacin, CIP	0.25 - 2
Erythromycin, ERY	1 - 4
Florfenicol, FFN	2 - 8
Gentamicin, GEN	4 - 16
Linezolid, LZD	1 - 4
Synacid, SYN	2 - 8
Tetracycline, TET	8 - 32
Vancomycin, VAN	1 - 4

	S. aureus ATCC 25923		*S. aureus* ATCC 29213
Antimicrobial	**Disk diffusion**	**ROSCO**	**MIC**
Chloramphenicol, CHL	16 - 26	None	2 - 8
Ciprofloxacin, CIP	22 - 30	21 - 29	0.12 - 0.5
Erythromycin, ERY	22 - 30	26 - 33	0.25 - 1
Florfenicol, FFN	None	None	2 - 8
Gentamicin, GEN	19 - 27	25 - 32	0.12 - 1
Penicillin, PEN	26 - 37	None	0.25 - 2
Streptomycin, STR	14 - 22	None	None
Suphonamides, SMX	24 - 30	26 - 34	32 - 128
Tetracycline, TET	24 - 34	23 - 33	0.12 - 1
Trimethoprim, TMP	19 - 26	19 - 25	1-4

E. coli ATCC 25922		
Antimicrobial	**Disk difusion**	**MIC**
Amoxicillin cl., AUG	18 - 24	2 - 8
Ampicillin, AMP	16 - 22	2 - 8
Cefotaxime, CTX	29 - 35	0.03 - 0.12
Ceftazidime, CAZ	25 - 32	0.06 - 0.5
Ceftiofur, XNL	26 - 31	0.25 - 1
Chloramphenicol, CHL	21 - 27	2 - 8
Ciprofloxacin, CIP	30 - 40	0.004 - 0.016
Florphenicol, FFN	22 - 28	2 - 8
Gentamicin, GEN	19 - 26	0.25 - 1
Nalidixic acid, NAL	22 - 28	1 - 4
Streptomycin, STR	None	4 - 16
Sulphonamides, SMX	15 - 23	8 - 32
Tetracycline, TET	18 - 25	0.5 - 2
Trimethoprim, TMP	21 - 28	0.5 - 2

MIC ranges and disc diffusion ranges are according to CLSI M100-S19 with one exception: The MIC range for streptomycin is according to Sensititre. Additionally, the range for ciprofloxacin is extended to include 0.016 as well.

Percentage of resistant and sensitive enterococci

Strain	Antimicrobial	Expected result	%R	%S	Number expected results	Number deviating results
EURL ENT.4,1	Ampicillin , AMP	S	15	85	17	3
	Chloramphenicol, CHL	S	5	95	21	1
	Ciprofloxacin , CIP	S	0	100	18	0
	Erythromycin, ERY	R	100	0	22	0
	Gentamicin, GEN	S	5	95	21	1
	Linezolid, LZD	S	0	100	19	0
	Streptomycin, STR	R	100	0	22	0
	Synacid, SYN	R	100	0	11	0
	Tetracycline, TET	R	100	0	22	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**195**	**5**
EURL ENT.4,2	Ampicillin , AMP	S	0	100	20	0
	Chloramphenicol, CHL	S	0	100	22	0
	Ciprofloxacin , CIP	S	0	100	18	0
	Erythromycin, ERY	R	95	5	21	1
	Gentamicin, GEN	R	95	5	21	1
	Linezolid, LZD	S	0	100	18	0
	Streptomycin, STR	R	95	5	20	1
	Synacid, SYN	S	20	80	8	2
	Tetracycline, TET	R	95	5	21	1
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**191**	**6**
EURL ENT.4,3	Ampicillin , AMP	S	0	100	20	0
	Chloramphenicol, CHL	R	100	0	22	0
	Ciprofloxacin , CIP	S	0	100	18	0
	Erythromycin, ERY	R	100	0	22	0
	Gentamicin, GEN	S	14	86	19	3
	Linezolid, LZD	S	5	95	18	1
	Streptomycin, STR	R	100	0	21	0
	Synacid, SYN	S	20	80	8	2
	Tetracycline, TET	R	100	0	22	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**192**	**6**
EURL ENT.4,4	Ampicillin , AMP	S	0	100	20	0
	Chloramphenicol, CHL	R	100	0	22	0
	Ciprofloxacin , CIP	S	0	100	18	0
	Erythromycin, ERY	R	100	0	22	0
	Gentamicin, GEN	R	100	0	22	0
	Linezolid, LZD	S	0	100	18	0
	Streptomycin, STR	R	100	0	21	0
	Synacid, SYN	S	20	80	8	2
	Tetracycline, TET	R	100	0	22	0
	Vancomycin, VAN	S	5	95	21	1
	TOTAL				**194**	**3**

Strain	Antimicrobial	Expected result	%R	%S	Number expected results	Number deviating results
EURL ENT.4,5	Ampicillin , AMP	S	0	100	20	0
	Chloramphenicol, CHL	S	0	100	22	0
	Ciprofloxacin , CIP	S	11	89	16	2
	Erythromycin, ERY	S	9	91	20	2
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	0	100	19	0
	Streptomycin, STR	S	5	95	21	1
	Synacid, SYN	S	20	80	8	2
	Tetracycline, TET	S	0	100	22	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**192**	**7**
EURL ENT.4,6	Ampicillin , AMP	R	20	80	4	16
	Chloramphenicol, CHL	S	5	95	21	1
	Ciprofloxacin , CIP	S	0	100	18	0
	Erythromycin, ERY	R	100	0	22	0
	Gentamicin, GEN	S	5	95	21	1
	Linezolid, LZD	S	0	100	19	0
	Streptomycin, STR	R	100	0	22	0
	Synacid, SYN	R	100	0	11	0
	Tetracycline, TET	R	100	0	22	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**182**	**18**
EURL ENT.4,7	Ampicillin , AMP	S	10	90	18	2
	Chloramphenicol, CHL	S	5	95	21	1
	Ciprofloxacin , CIP	S	0	100	18	0
	Erythromycin, ERY	R	100	0	22	0
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	5	95	18	1
	Streptomycin, STR	R	100	0	22	0
	Synacid, SYN	R	100	0	11	0
	Tetracycline, TET	R	100	0	22	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**194**	**6**
EURL ENT.4,8	Ampicillin , AMP	S	10	90	18	2
	Chloramphenicol, CHL	S	5	95	21	1
	Ciprofloxacin , CIP	S	6	94	17	1
	Erythromycin, ERY	S	5	95	21	1
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	5	95	18	1
	Streptomycin, STR	S	10	90	19	2
	Synacid, SYN	R	100	0	11	0
	Tetracycline, TET	R	100	0	22	0
	Vancomycin, VAN	R	95	5	21	1
	TOTAL				**188**	**11**

Antimicrobials producing deviations

Percentage of resistant and sensitive staphylococci

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
EURL ST.4,1	Cefoxitin, FOX	R	95	5	19	1
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	4	96	26	1
	Erythromycin, ERY	R	96	4	27	1
	Florfenicol, FFN	S	0	100	15	0
	Gentamicin, GEN	S	0	100	25	0
	Penicillin, PEN	R	100	0	25	0
	Streptomycin, STR	R	100	0	23	0
	Sulfamethoxazole, SMX	S	6	94	17	1
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	S	9	91	21	2
	TOTAL				**251**	**6**
EURL ST.4,2	Cefoxitin, FOX	S	0	100	20	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	R	56	44	15	12
	Erythromycin, ERY	R	96	4	27	1
	Florfenicol, FFN	S	0	100	14	0
	Gentamicin, GEN	S	0	100	26	0
	Penicillin, PEN	R	96	4	24	1
	Streptomycin, STR	S	0	100	23	0
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	S	4	96	22	1
	TOTAL				**242**	**15**
EURL ST.4,3	Cefoxitin, FOX	S	0	100	20	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	0	100	27	0
	Erythromycin, ERY	S	4	96	27	1
	Florfenicol, FFN	S	0	100	14	0
	Gentamicin, GEN	R	100	0	27	0
	Penicillin, PEN	R	96	4	24	1
	Streptomycin, STR	S	4	96	22	1
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	S	0	100	28	0
	Trimethoprim, TMP	R	100	0	23	0
	TOTAL				**255**	**3**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
EURL ST.4,4	Cefoxitin, FOX	R	100	0	20	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	0	100	27	0
	Erythromycin, ERY	S	4	96	27	1
	Florfenicol, FFN	S	0	100	14	0
	Gentamicin, GEN	S	4	96	25	1
	Penicillin, PEN	R	100	0	25	0
	Streptomycin, STR	R	100	0	23	0
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	R	100	0	23	0
	TOTAL				**255**	**2**
EURL ST.4,5	Cefoxitin, FOX	R	100	0	19	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	R	41	59	11	16
	Erythromycin, ERY	S	0	100	28	0
	Florfenicol, FFN	S	0	100	14	0
	Gentamicin, GEN	R	100	0	27	0
	Penicillin, PEN	R	100	0	25	0
	Streptomycin, STR	R	100	0	23	0
	Sulfamethoxazole, SMX	R	84	16	16	3
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	S	0	100	23	0
	TOTAL				**239**	**19**
EURL ST.4,6	Cefoxitin, FOX	S	0	100	20	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	0	100	27	0
	Erythromycin, ERY	S	0	100	28	0
	Florfenicol, FFN	S	0	100	14	0
	Gentamicin, GEN	S	0	100	26	0
	Penicillin, PEN	R	96	4	24	1
	Streptomycin, STR	R	100	0	23	0
	Sulfamethoxazole, SMX	S	0	100	19	0
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	R	100	0	23	0
	TOTAL				**257**	**1**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
EURL ST.4,7	Cefoxitin, FOX	S	5	95	19	1
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	0	100	27	0
	Erythromycin, ERY	S	0	100	28	0
	Florfenicol, FFN	S	0	100	14	0
	Gentamicin, GEN	S	0	100	26	0
	Penicillin, PEN	S	4	96	24	1
	Streptomycin, STR	S	0	100	23	0
	Sulfamethoxazole, SMX	S	0	100	19	0
	Tetracycline, TET	S	0	100	28	0
	Trimethoprim, TMP	S	4	96	22	1
	TOTAL				**255**	**3**
EURL ST.4,8	Cefoxitin, FOX	S	0	100	20	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	4	96	26	1
	Erythromycin, ERY	S	0	100	28	0
	Florfenicol, FFN	S	0	100	14	0
	Gentamicin, GEN	S	0	100	26	0
	Penicillin, PEN	R	96	4	24	1
	Streptomycin, STR	S	0	100	23	0
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	R	100	0	23	0
	TOTAL				**255**	**2**

Antimicrobials producing deviations

Percentage of resistant and sensitive *E. coli*

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
EURL EC.4,1	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	S	7	93	27	2
	Ceftazidime, CAZ	S	4	96	23	1
	Ceftiofur, XNL	S	11	89	8	1
	Chloramphenicol, CHL	R	97	3	28	1
	Ciprofloxacin, CIP	S	0	100	29	0
	Florphenicol, FFN	R	100	0	24	0
	Gentamicin, GEN	S	3	97	28	1
	Nalidixic acid, NAL	S	0	100	29	0
	Streptomycin, STR	R	96	4	27	1
	Sulfamethoxazole, SMX	R	96	4	27	1
	Tetracycline, TET	S	3	97	28	1
	Trimethoprim, TMP	S	4	96	26	1
	TOTAL				**333**	**10**
EURL EC.4,2	Ampicillin, AMP	S	0	100	29	0
	Cefotaxime, CTX	S	0	100	29	0
	Ceftazidime, CAZ	S	0	100	24	0
	Ceftiofur, XNL	S	0	100	9	0
	Chloramphenicol, CHL	S	0	100	29	0
	Ciprofloxacin, CIP	S	0	100	29	0
	Florphenicol, FFN	S	0	100	24	0
	Gentamicin, GEN	S	0	100	29	0
	Nalidixic acid, NAL	S	0	100	29	0
	Streptomycin, STR	S	0	100	27	0
	Sulfamethoxazole, SMX	S	4	96	27	1
	Tetracycline, TET	S	3	97	28	1
	Trimethoprim, TMP	S	0	100	27	0
	TOTAL				**340**	**2**
EURL EC.4,3	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	S	0	100	29	0
	Ceftazidime, CAZ	S	0	100	24	0
	Ceftiofur, XNL	S	11	89	8	1
	Chloramphenicol, CHL	S	0	100	29	0
	Ciprofloxacin, CIP	S	0	100	29	0
	Florphenicol, FFN	S	4	96	23	1
	Gentamicin, GEN	S	0	100	29	0
	Nalidixic acid, NAL	S	0	100	29	0
	Streptomycin, STR	R	100	0	28	0
	Sulfamethoxazole, SMX	R	100	0	28	0
	Tetracycline, TET	R	93	7	27	2
	Trimethoprim, TMP	R	96	4	26	1
	TOTAL				**338**	**5**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
EURL EC.4,4	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	S	0	100	29	0
	Ceftazidime, CAZ	S	0	100	24	0
	Ceftiofur, XNL	S	10	90	9	1
	Chloramphenicol, CHL	S	3	97	28	1
	Ciprofloxacin, CIP	R	97	3	28	1
	Florphenicol, FFN	S	4	96	23	1
	Gentamicin, GEN	S	3	97	28	1
	Nalidixic acid, NAL	R	100	0	29	0
	Streptomycin, STR	R	100	0	28	0
	Sulfamethoxazole, SMX	R	96	4	27	1
	Tetracycline, TET	R	97	3	28	1
	Trimethoprim, TMP	S	0	100	27	0
	TOTAL				**337**	**7**
EURL EC.4,5	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	R	100	0	29	0
	Ceftazidime, CAZ	R	96	4	23	1
	Ceftiofur, XNL	R	89	11	8	1
	Chloramphenicol, CHL	S	3	97	28	1
	Ciprofloxacin, CIP	S	0	100	29	0
	Florphenicol, FFN	S	0	100	24	0
	Gentamicin, GEN	S	0	100	29	0
	Nalidixic acid, NAL	S	0	100	29	0
	Streptomycin, STR	S	0	100	27	0
	Sulfamethoxazole, SMX	S	4	96	27	1
	Tetracycline, TET	S	0	100	29	0
	Trimethoprim, TMP	S	0	100	27	0
	TOTAL				**338**	**4**
EURL EC.4,6	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	S	0	100	29	0
	Ceftazidime, CAZ	S	0	100	24	0
	Ceftiofur, XNL	S	11	89	8	1
	Chloramphenicol, CHL	R	97	3	28	1
	Ciprofloxacin, CIP	R	97	3	28	1
	Florphenicol, FFN	S	17	83	20	4
	Gentamicin, GEN	S	7	93	27	2
	Nalidixic acid, NAL	S	7	93	27	2
	Streptomycin, STR	R	58	42	15	11
	Sulfamethoxazole, SMX	R	100	0	28	0
	Tetracycline, TET	R	100	0	29	0
	Trimethoprim, TMP	R	100	0	27	0
	TOTAL				**319**	**22**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
EURL EC.4,7	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	R	97	3	28	1
	Ceftazidime, CAZ	R	96	4	23	1
	Ceftiofur, XNL	R	78	22	7	2
	Chloramphenicol, CHL	S	7	93	27	2
	Ciprofloxacin, CIP	R	93	7	27	2
	Florphenicol, FFN	S	4	96	23	1
	Gentamicin, GEN	S	0	100	29	0
	Nalidixic acid, NAL	R	97	3	28	1
	Streptomycin, STR	R	100	0	28	0
	Sulfamethoxazole, SMX	R	100	0	28	0
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	R	100	0	28	0
	TOTAL				**330**	**13**
EURL EC.4,8	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	R	90	10	26	3
	Ceftazidime, CAZ	R	92	8	22	2
	Ceftiofur, XNL	R	100	0	9	0
	Chloramphenicol, CHL	R	97	3	28	1
	Ciprofloxacin, CIP	R	100	0	29	0
	Florphenicol, FFN	R	67	33	16	8
	Gentamicin, GEN	R	90	10	26	3
	Nalidixic acid, NAL	R	100	0	29	0
	Streptomycin, STR	R	100	0	28	0
	Sulfamethoxazole, SMX	R	100	0	28	0
	Tetracycline, TET	R	100	0	29	0
	Trimethoprim, TMP	R	100	0	27	0
	TOTAL				**326**	**17**

Antimicrobials producing deviations

Deviations per laboratory for the enterococci strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
15	EURL ENT.4,5	Ciprofloxacin , CIP	R	16	S	2	DD
18	EURL ENT.4,2	Synacid, SYN	R	10	S	16	DD
	EURL ENT.4,3	Gentamicin, GEN	R	11	S	16	
	EURL ENT.4,3	Synacid, SYN	R	12	S	8	
	EURL ENT.4,4	Synacid, SYN	R	11	S	16	
	EURL ENT.4,5	Gentamicin, GEN	R	12	S	16	
	EURL ENT.4,5	Streptomycin, STR	R	6	S	64	
	EURL ENT.4,5	Synacid, SYN	R	13	S	8	
19	EURL ENT.4,3	Gentamicin, GEN	R	1024	S	16	MIC
20	EURL ENT.4,7	Ampicillin , AMP	R	8	S	4	MIC
24	EURL ENT.4,1	Ampicillin , AMP	R	8	S	4	MIC
26	EURL ENT.4,1	Chloramphenicol, CHL	R	9	S	8	DD
	EURL ENT.4,1	Gentamicin, GEN	R	6	S	16	
	EURL ENT.4,2	Synacid, SYN	R	12	S	16	
	EURL ENT.4,3	Gentamicin, GEN	R	10	S	16	
	EURL ENT.4,3	Synacid, SYN	R	12	S	8	
	EURL ENT.4,4	Synacid, SYN	R	13	S	16	
	EURL ENT.4,5	Ciprofloxacin , CIP	R	14	S	2	
	EURL ENT.4,5	Gentamicin, GEN	R	9	S	16	
	EURL ENT.4,5	Synacid, SYN	R	13	S	8	
	EURL ENT.4,6	Chloramphenicol, CHL	R	9	S	8	
	EURL ENT.4,6	Gentamicin, GEN	R	6	S	16	
	EURL ENT.4,7	Chloramphenicol, CHL	R	10	S	8	
	EURL ENT.4,7	Gentamicin, GEN	R	6	S	16	
	EURL ENT.4,8	Chloramphenicol, CHL	R	10	S	4	
	EURL ENT.4,8	Erythromycin, ERY	R	6	S	2	
	EURL ENT.4,8	Gentamicin, GEN	R	6	S	32	
	EURL ENT.4,8	Streptomycin, STR	R	6	S	64	
	EURL ENT.4,8	Vancomycin, VAN	S	16	R	>32	
34	EURL ENT.4,4	Vancomycin, VAN	R	8	S	4	MIC
37	EURL ENT.4,1	Ampicillin , AMP	R	8	S	4	MIC
	EURL ENT.4,7	Ampicillin , AMP	R	8	S	4	
	EURL ENT.4,8	Ampicillin , AMP	R	16	S	4	
39	EURL ENT.4,2	Erythromycin, ERY	S	<0.5	R	>32	MIC
	EURL ENT.4,2	Gentamicin, GEN	S	<2	R	512	
	EURL ENT.4,2	Streptomycin, STR	S	<8	R	>2048	
	EURL ENT.4,2	Tetracycline, TET	S	<0.5	R	>32	
40	EURL ENT.4,1	Ampicillin , AMP	R	10	S	4	DD
	EURL ENT.4,3	Linezolid, LZD	R	20	S	1	
	EURL ENT.4,7	Gentamicin, GEN	R	12	S	16	
	EURL ENT.4,7	Linezolid, LZD	R	17	S	2	
	EURL ENT.4,8	Ampicillin , AMP	R	6	S	4	
	EURL ENT.4,8	Ciprofloxacin , CIP	R	12	S	1	
	EURL ENT.4,8	Gentamicin, GEN	R	6	S	32	
	EURL ENT.4,8	Linezolid, LZD	R	16	S	2	
	EURL ENT.4,8	Streptomycin, STR	R	8	S	64	
44	EURL ENT.4,6	Ampicillin , AMP	S	4	R	8	MIC

Deviations per laboratory for the staphylococci strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
13	EURL ST.4,1	Erythromycin, ERY	S	13	R	>16	DD
	EURL ST.4,2	Erythromycin, ERY	S	14	R	>16	
	EURL ST.4,5	Sulfamethoxazole, SMX	S	12	R	512	
14	EURL ST.4,1	Ciprofloxacin, CIP	R	21	S	0,5	DD
	EURL ST.4,8	Ciprofloxacin, CIP	R	21	S	1	
21	EURL ST.4,2	Penicillin, PEN	S	0.5	R	2	MIC
	EURL ST.4,3	Penicillin, PEN	S	2	R	8	
	EURL ST.4,6	Penicillin, PEN	S	1	R	2	
	EURL ST.4,8	Penicillin, PEN	S	0.5	R	4	
22	EURL ST.4,3	Erythromycin, ERY	R	2	S	0,5	MIC
23	EURL ST.4,1	Cefoxitin, FOX	S	4	R	8	MIC
24	EURL ST.4,3	Streptomycin, STR	R	<8	S	8	MIC
30	EURL ST.4,1	Sulfamethoxazole, SMX	R	256	S	32	MIC
	EURL ST.4,1	Trimethoprim, TMP	R	4	S	0,5	
	EURL ST.4,4	Erythromycin, ERY	R	>4	S	0,5	
	EURL ST.4,5	Sulfamethoxazole, SMX	S	128	R	512	
33	EURL ST.4,7	Cefoxitin, FOX	R	8	S	2	MIC
34	EURL ST.4,5	Sulfamethoxazole, SMX	S	128	R	512	MIC
39	EURL ST.4,1	Trimethoprim, TMP	R	4	S	0,5	MIC
	EURL ST.4,2	Trimethoprim, TMP	R	4	S	0,5	
	EURL ST.4,7	Trimethoprim, TMP	R	4	S	0,5	
40	EURL ST.4,4	Gentamicin, GEN	R	6	S	0,25	DD
44	EURL ST.4,7	Penicillin, PEN	R	0.5	S	0,06	MIC

Deviations per laboratory for the *E. coli* strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
2	EURL EC.4,8	Cefotaxime, CTX	S	0.12	R	>4	MIC
	EURL EC.4,8	Gentamicin, GEN	S	<0.25	R	>16	
4	EURL EC.4,6	Florphenicol, FFN	R	16	S	16	MIC
11	EURL EC.4,6	Gentamicin, GEN	R	4	S	4	MIC
14	EURL EC.4,4	Chloramphenicol, CHL	R	18	S	8	DD
	EURL EC.4,6	Nalidixic acid, NAL	R	14	S	16	
	EURL EC.4,7	Ciprofloxacin, CIP	S	23	R	0,25	
15	EURL EC.4,6	Florphenicol, FFN	R	15	S	16	DD
	EURL EC.4,6	Nalidixic acid, NAL	R	11	S	16	
18	EURL EC.4,6	Florphenicol, FFN	R	14	S	16	DD
19	EURL EC.4,7	Nalidixic acid, NAL	S	64	R	>64	MIC
	EURL EC.4,8	Gentamicin, GEN	S	1	R	>16	
21	EURL EC.4,7	Chloramphenicol, CHL	R	32	S	4	MIC
	EURL EC.4,7	Trimethoprim, TMP	R	>32	S	1	
29	EURL EC.4,2	Tetracycline, TET	R	2	S	2	MIC
	EURL EC.4,3	Tetracycline, TET	S	<1	R	>32	
	EURL EC.4,7	Ceftiofur, XNL	S	21	R	8	
32	EURL EC.4,8	Cefotaxime, CTX	S	<0.12	R	>4	MIC
	EURL EC.4,8	Ceftazidime, CAZ	S	<0.25	R	32	
	EURL EC.4,8	Gentamicin, GEN	S	<0.5	R	>16	
37	EURL EC.4,6	Streptomycin, STR	S	16	R	32	MIC
39	EURL EC.4,1	Cefotaxime, CTX	R	1	S	0,125	MIC
	EURL EC.4,1	Ceftiofur, XNL	R	16	S	0,5	
	EURL EC.4,1	Chloramphenicol, CHL	S	2	R	>64	
	EURL EC.4,1	Gentamicin, GEN	R	4	S	1	
	EURL EC.4,1	Streptomycin, STR	S	<2	R	128	
	EURL EC.4,1	Sulfamethoxazole, SMX	S	64	R	>1024	
	EURL EC.4,1	Tetracycline, TET	R	64	S	2	
	EURL EC.4,1	Trimethoprim, TMP	R	32	S	1	
	EURL EC.4,3	Ceftiofur, XNL	R	16	S	0,5	
	EURL EC.4,3	Florphenicol, FFN	R	>32	S	4	
	EURL EC.4,3	Tetracycline, TET	S	<0.5	R	>32	
	EURL EC.4,3	Trimethoprim, TMP	S	1	R	>32	
	EURL EC.4,4	Ceftiofur, XNL	R	>16	S	0,5	
	EURL EC.4,4	Florphenicol, FFN	R	>32	S	8	
	EURL EC.4,4	Gentamicin, GEN	R	4	S	0,5	
	EURL EC.4,4	Sulfamethoxazole, SMX	S	64	R	>1024	
	EURL EC.4,4	Tetracycline, TET	S	<0.5	R	>32	
	EURL EC.4,5	Ceftiofur, XNL	S	0.25	R	>8	
	EURL EC.4,5	Chloramphenicol, CHL	R	>128	S	4	
	EURL EC.4,6	Ceftiofur, XNL	R	4	S	0,5	
	EURL EC.4,6	Chloramphenicol, CHL	S	4	R	>64	
	EURL EC.4,6	Florphenicol, FFN	R	>32	S	16	
	EURL EC.4,6	Gentamicin, GEN	R	16	S	4	
	EURL EC.4,7	Chloramphenicol, CHL	R	>128	S	4	
	EURL EC.4,7	Florphenicol, FFN	R	>32	S	4	
	EURL EC.4,7	Sulfamethoxazole, SMX	S	64	R	>1024	
	EURL EC.4,7	Tetracycline, TET	S	<0.5	R	>32	
	EURL EC.4,8	Cefotaxime, CTX	S	0.25	R	>4	
	EURL EC.4,8	Chloramphenicol, CHL	S	8	R	>64	

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
40	EURL EC.4,2	Sulfamethoxazole, SMX	R	6	S	64	DD
	EURL EC.4,4	Ciprofloxacin, CIP	S	28	R	0,5	
	EURL EC.4,5	Ceftazidime, CAZ	S	24	R	2	
	EURL EC.4,6	Ciprofloxacin, CIP	S	22	R	0,5	
	EURL EC.4,7	Cefotaxime, CTX	S	22	R	>4	
	EURL EC.4,7	Ceftazidime, CAZ	S	18	R	16	
	EURL EC.4,7	Ceftiofur, XNL	S	17	R	8	
	EURL EC.4,7	Ciprofloxacin, CIP	S	28	R	0,25	
	EURL EC.4,8	Ceftazidime, CAZ	S	17	R	32	
44	EURL EC.4,1	Cefotaxime, CTX	R	2	S	0,125	MIC
	EURL EC.4,1	Ceftazidime, CAZ	R	1	S	0,25	
	EURL EC.4,5	Sulfamethoxazole, SMX	R	>1024	S	64	

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